



**University of  
Zurich**<sup>UZH</sup>

**Zurich Open Repository and  
Archive**

University of Zurich  
University Library  
Strickhofstrasse 39  
CH-8057 Zurich  
[www.zora.uzh.ch](http://www.zora.uzh.ch)

---

Year: 2017

---

## Biological evaluation of pyridone alkaloids on the endocannabinoid system

Chicca, Andrea ; Berg, Regina ; Jessen, Henning J ; Marck, Nicolas ; Schmid, Fabian ; Burch, Patrick ;  
Gertsch, Jürg ; Gademann, Karl

**Abstract:** Naturally occurring pyridone alkaloids as well as synthetic derivatives were previously shown to induce neurite outgrowth. However, the molecular basis for this biological effect remains poorly understood. In this work, we have prepared new pyridones, and tested the effect of thirteen 4-hydroxy-2-pyridone derivatives on the components of the endocannabinoid system. Investigation of binding affinities towards CB1 and CB2 receptors led to the identification of a compound binding selectively to CB1 (12). Compound 12 and a closely related derivative (11) also inhibited anandamide (AEA) hydrolysis by fatty acid amide hydrolase. Interestingly, none of the compounds tested showed any effect on 2-AG hydrolysis by monoacylglycerol lipase at 10<sup>-6</sup> M. Assessment of AEA uptake did, however, lead to the identification of four inhibitors with IC<sub>50</sub> values in the submicromolar range and high selectivity over the other components of the endocannabinoid system.

DOI: <https://doi.org/10.1016/j.bmc.2017.02.031>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-150834>

Journal Article

Accepted Version



The following work is licensed under a Creative Commons: Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.

Originally published at:

Chicca, Andrea; Berg, Regina; Jessen, Henning J; Marck, Nicolas; Schmid, Fabian; Burch, Patrick; Gertsch, Jürg; Gademann, Karl (2017). Biological evaluation of pyridone alkaloids on the endocannabinoid system. *Bioorganic Medicinal Chemistry*, 25(22):6102-6114.

DOI: <https://doi.org/10.1016/j.bmc.2017.02.031>

## Biological Evaluation of Pyridone Alkaloids on the Endocannabinoid System

Andrea Chicca<sup>a</sup>, Regina Berg<sup>b</sup>, Henning J. Jessen<sup>c</sup>, Nicolas Marck<sup>b</sup>, Fabian Schmid<sup>c</sup>, Patrick Burch<sup>c</sup>, Jürg Gertsch<sup>a\*</sup>, Karl Gademann<sup>b\*</sup>

<sup>a</sup> NCCR-TransCure, Institute of Biochemistry and Molecular Medicine, University of Bern, Buehlstrasse 28, CH-3012 Bern, Switzerland.

<sup>b</sup> Department of Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.

<sup>c</sup> Department of Chemistry, University of Basel, St. Johannis-Ring 19, CH-4056 Basel, Switzerland.

\* Corresponding authors. *E-mail addresses:* juerg.gertsch@ibmm.unibe.ch (Prof. Dr. J. Gertsch); karl.gademann@uzh.ch (Prof. Dr. K. Gademann).

## Abstract

Naturally occurring pyridone alkaloids as well as synthetic derivatives were previously shown to induce neurite outgrowth. However, the molecular basis for this biological effect remains poorly understood. In this work, we have prepared new pyridones, and tested the effect of thirteen 4-hydroxy-2-pyridone derivatives on the components of the endocannabinoid system. Investigation of binding affinities towards CB<sub>1</sub> and CB<sub>2</sub> receptors led to the identification of a compound binding selectively to CB<sub>1</sub> (**12**). Compound **12** and a closely related derivative (**11**) also inhibited anandamide (AEA) hydrolysis by fatty acid amide hydrolase. Interestingly, none of the compounds tested showed any effect on 2-AG hydrolysis by monoacylglycerol lipase at 10  $\mu$ M. Assessment of AEA uptake did, however, lead to the identification of four inhibitors with IC<sub>50</sub> values in the submicromolar range and high selectivity over the other components of the endocannabinoid system.

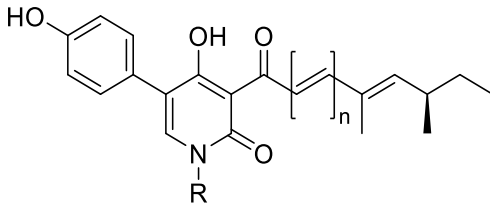
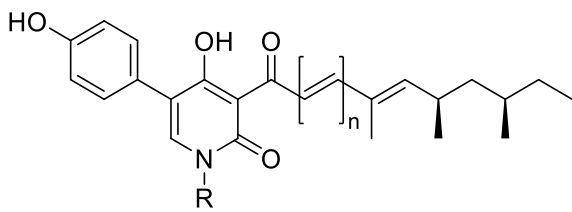
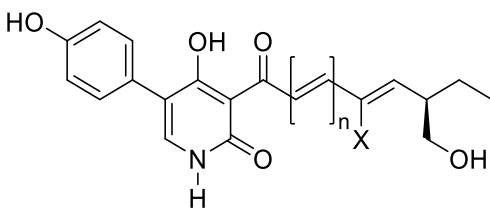
## 1. Introduction

Hydroxy-2-pyridones constitute a compelling class of secondary metabolites,<sup>[1]</sup> which have been suggested to act as chemical mediators in interactions between entomopathogenic fungi and their insect hosts. Over the past years, a number of biological activities have been reported for these compounds, mostly focusing on their *in vitro* properties, such as cytotoxicity.<sup>[2]</sup> For example, ricinine, a 2-pyridone alkaloid first isolated from castor oil plants in the 19<sup>th</sup> century,<sup>[3]</sup> has long been known as an insecticide<sup>[4]</sup> and lately been reported to have stimulatory effects on the central nervous system.<sup>[5]</sup> Pronounced antifungal activity has been observed with the structurally more complex compounds ilicicolin H,<sup>[2d, 6]</sup> apiosporamide,<sup>[7]</sup> and YM-215343.<sup>[2e]</sup> However, the molecular targets of 4-hydroxy-2-pyridones have generally remained elusive.<sup>[8]</sup> A recent study by the group of Waldmann, however, demonstrated an inhibitory effect on kinase activity, and a crystal structure of 3-acyl-4-hydroxy-2-pyridone complexed with wild-type mitogen-activated protein kinase 4 (MAP4K4) with the inhibitor residing in the ATP binding site supports this hypothesis.<sup>[9]</sup>

There have been numerous efforts directed towards the total synthesis of 4-hydroxy-2-pyridones, as it is difficult to obtain these alkaloids in pure form from natural sources.<sup>[10]</sup> The work of one of our groups has focused on a family of 3-acyl-4-hydroxy-2-pyridone

alkaloids isolated from entomopathogenic fungi (Table 1).<sup>[2b, 11]</sup> Chemical synthesis yielded the congeners pretenellin B, prebassianin B, farinosone A, militarinone D and another homologue of the militarinone family (HJJ-510). All of these substances show neuritogenic activity in the PC12 cell assay.<sup>[12]</sup> Based on these phenotypic observations, we became interested in the potential mechanism of action of these compounds.

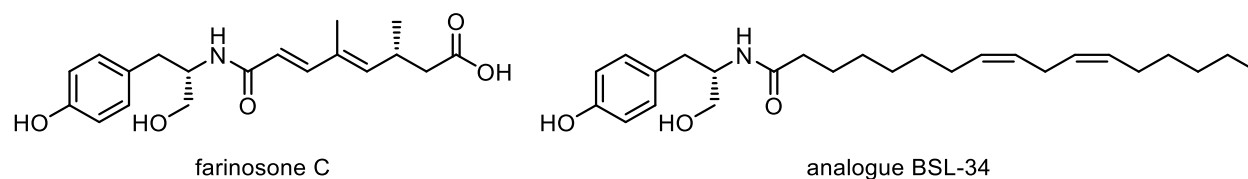
Table 1: Family of pyridone alkaloids from entomopathogenic fungi.

	R = H, n = 1	<b>pretenellin B</b>
	R = OH, n = 1	tenellin
	<b>R = H, n = 2</b>	<b>prebassianin B</b>
	R = OH, n = 2	bassianin
	<b>R = H, n = 3</b>	<b>farinosone A</b>
	R = OH, n = 3	farinosone B
	<b>R = H, n = 1</b>	<b>unknown (HJJ-510)</b>
	R = OH, n = 1	unknown
	<b>R = H, n = 2</b>	<b>militarinone D</b>
	R = OH, n = 2	unknown
	<b>X = CH<sub>3</sub>, n = 1</b>	<b>pyridovericin</b>
	<b>X = H, n = 2</b>	<b>torrubiellone C</b>

The structurally related compound farinosone C triggers neurite outgrowth, and we have shown that structurally simplified analogues retain this activity.<sup>[13]</sup> In order to investigate the underlying biochemical pathways responsible for neurite outgrowth, these substances were tested for their ability to bind to cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>). It had been previously reported that CB<sub>1</sub> receptor activation promotes neuritogenesis in Neuro-2A cells via a complex signalling pathway<sup>[14]</sup> and to restore neuritogenic activity in hyperglycemic PC12 cells.<sup>[15]</sup> One of the farinosone C analogues (BSL-34; Scheme 1) very effectively inhibits anandamide (AEA) uptake – most likely by interacting with the

putative endocannabinoid membrane transporter, which is supposed to regulate the bidirectional shuttle of endocannabinoids across the plasma membrane or via interaction with cytoplasmic shuttle proteins.<sup>[16]</sup>

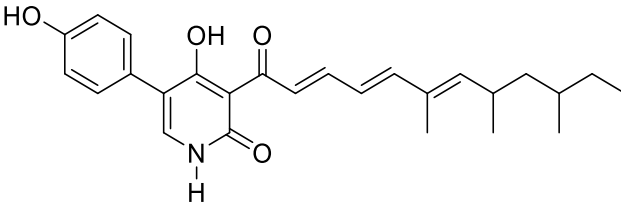
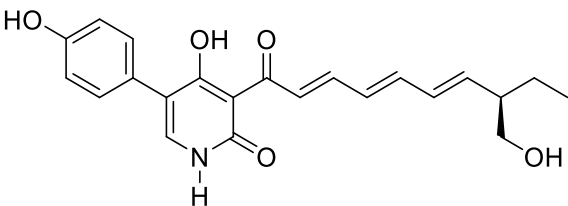
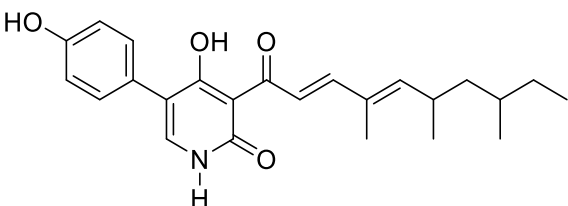
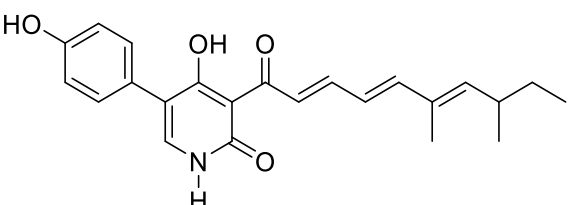
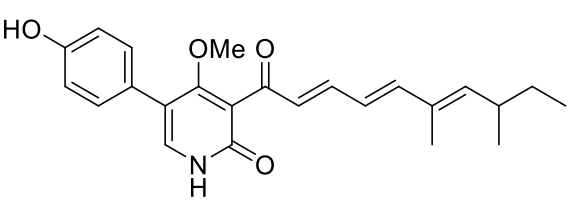
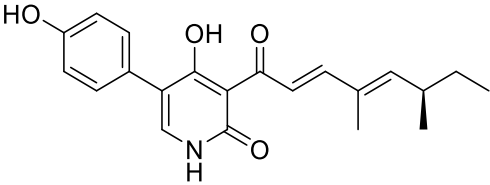
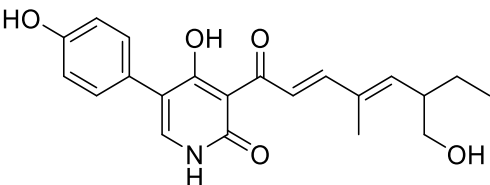
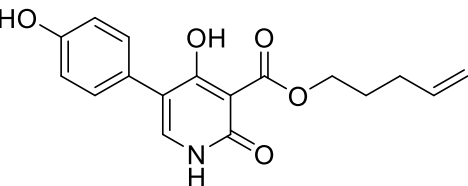
Scheme 1: Neuritogenic compounds farinosone C and BSL-34.



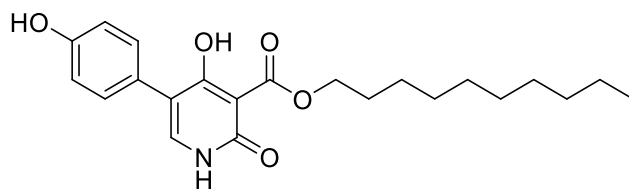
Based on these observations, we reasoned that AEA uptake inhibition might be the cause for the neuritogenic effects exhibited by BSL-34 because such inhibition would lead to an increase in the extracellular concentrations of AEA, which can in turn activate cannabinoid receptors. In addition, increasing levels of AEA might also affect TRPV1 activity, which plays a role in neuronal differentiation of SH-SY5Y human neuroblastoma cells.<sup>[17]</sup> Therefore, we investigated whether the neuritogenic effects of the pyridone alkaloids are related to activation of CB<sub>1</sub> and CB<sub>2</sub> and/or indirect modulation of AEA levels by inhibition of the degrading enzymes and/or specifically AEA uptake. All compounds tested – eight natural products, two truncated ester derivatives and three derivatives with ketone side chains of various length – are shown in Table 2.

Table 2. Chemical structure of the pyridone alkaloids tested on the different components of the ECS and known inhibitors for positive controls.

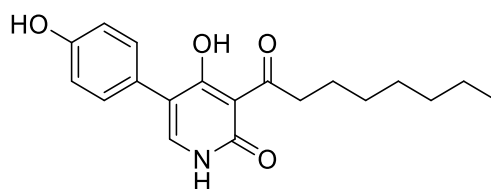
entry	name	structure
1	farinosone A	

2	militarinone D	
3	torrubiellone C	
4	HJJ-510	
5	prebassianin B	
6	SF-182	
7	pretenellin B	
8	pyridovericin	
9	NM33	

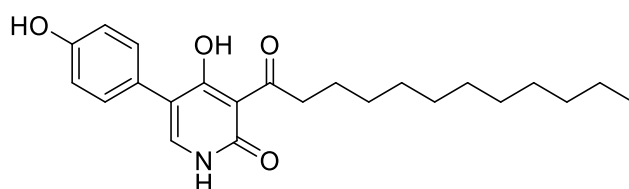
10 NM34/36



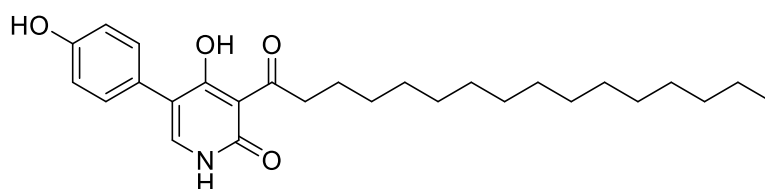
11 NM56



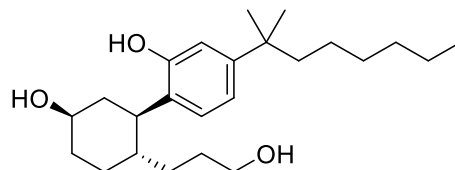
12 NM57



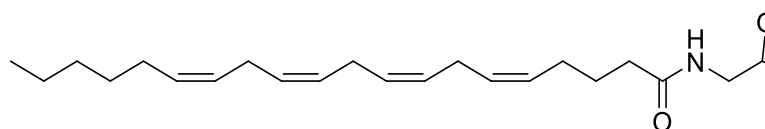
13 NM58



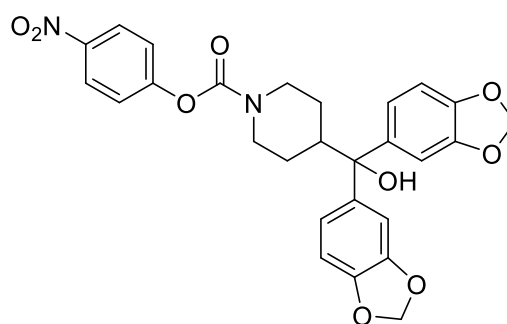
14 CP55940



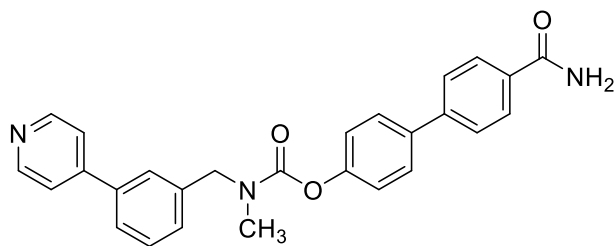
15 UCM707<sup>[18]</sup>



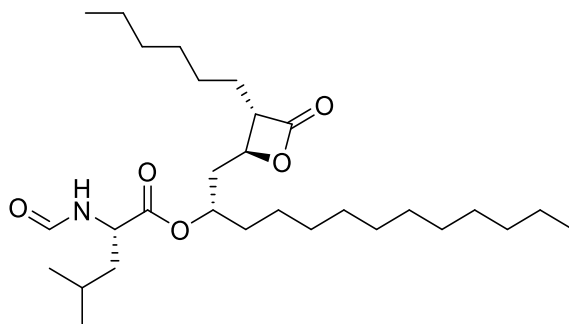
16 JZL184<sup>[19]</sup>



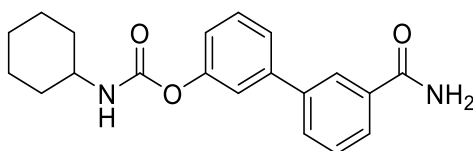
17 WWL70<sup>[20]</sup>



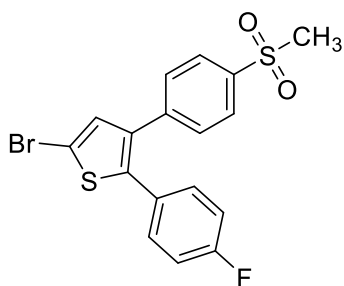
18 THL<sup>[20]</sup>



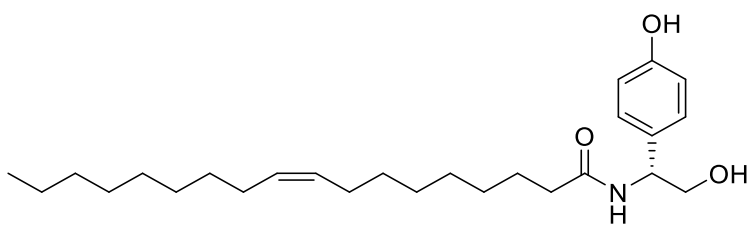
19 URB597<sup>[18, 21]</sup>



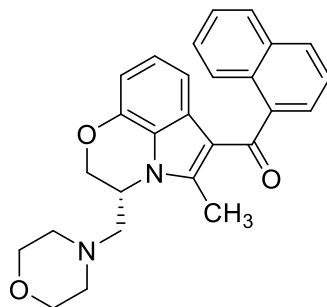
20 DuP697<sup>[22]</sup>



21 OMDM-2<sup>[23]</sup>



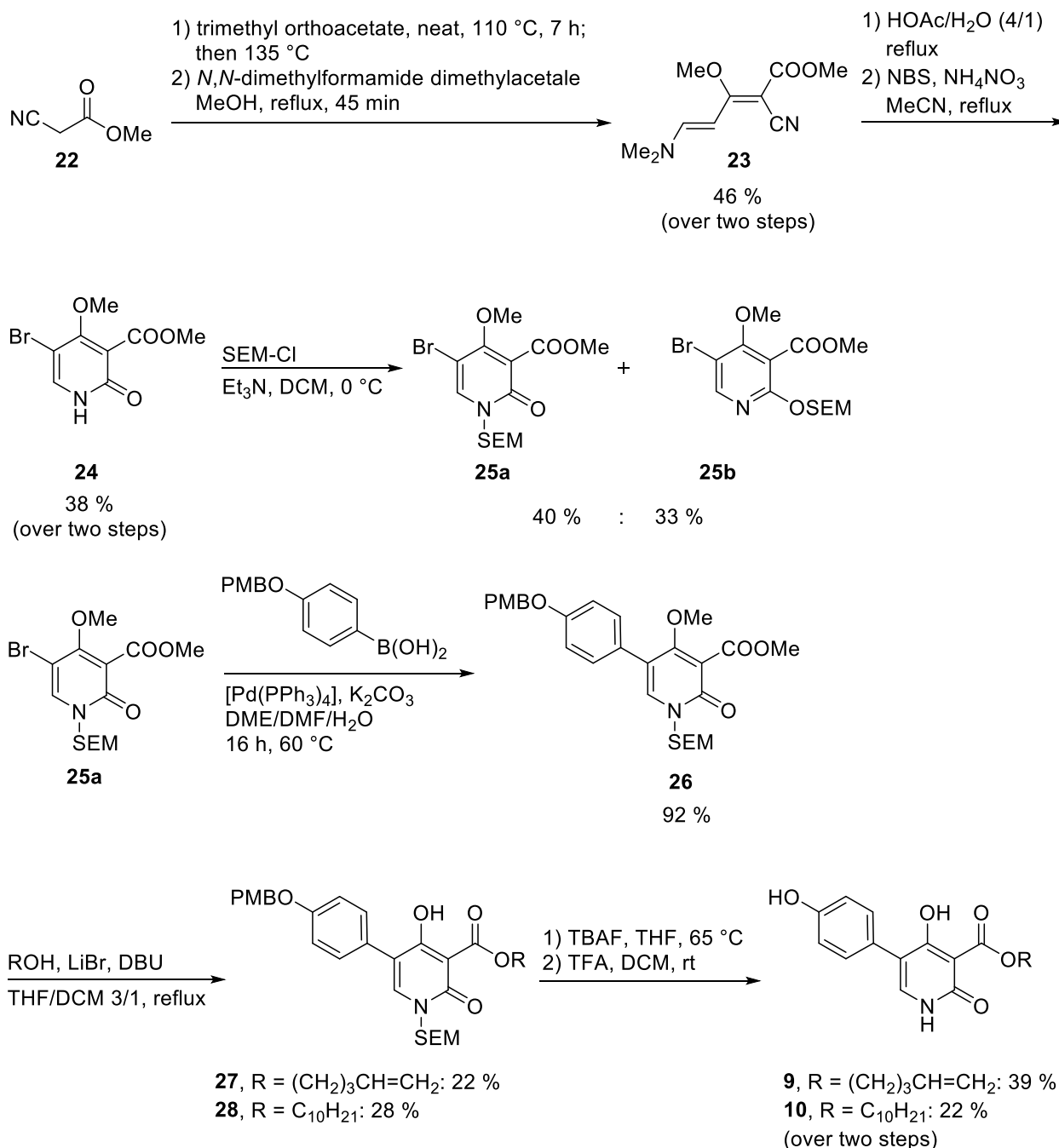
22 WIN55,212-2<sup>[24]</sup>



## 2. Chemistry



The natural products tested in this study for their activity towards components of the endocannabinoid system were obtained by total synthesis as reported in literature.<sup>[10i, 12, 25]</sup> As we aimed to produce 4-hydroxy-2-pyridone derivatives with diverse side chains at position C-3, we first envisioned a late-stage transacylation of the methyl ester analogue (**27**) of the known ethyl ester intermediate from the total synthesis of natural pyridone alkaloids.<sup>[12]</sup> We thus prepared methyl (Z)-2-cyano-3-methoxybut-2-enoate by condensation of methyl cyanoacetate (**22**) with trimethylorthoacetate according to a procedure first reported by Arndt in 1936 with concomitant distillation of methanol.<sup>[26]</sup> Upon cooling, the product solidified. It was re-crystallized from an aqueous methanol solution (62 % yield) and used without further purification in the subsequent condensation with *N,N*-dimethylformamide dimethyl acetal to give the bright yellow push-pull substituted olefin **23** with 74 % yield. Heating this enamine in acetic acid triggers nucleophilic attack of the amino group at the cyano group, finally leading to formation of the pyridine ring, as reported by the group of Prager.<sup>[27]</sup> However, due to the harsh reaction conditions this procedure is prone to side product formation and the isolated yield of the desired product was only 40 %. Subsequently, the pyridone ring was site-selectively brominated in 5-position with an excellent yield of 96 % to give methyl 5-bromo-4-methoxy-2-oxo-1,2-dihydropyridine-3-carboxylate **24**. As in the total synthesis of pyridone alkaloid natural products,<sup>[12]</sup> the pyridone ring was protected by reaction with 2-(trimethylsilyl)ethoxymethyl chloride giving the *N*-SEM (**25a**) and the *O*-SEM product (**25b**) in a ratio of 4:3. Both derivatives undergo facile Suzuki coupling with {4-[(4-methoxybenzyl)oxy]phenyl}-boronic acid, but in this study the *N*-SEM isomer was further utilized. With the PMB-protected 4-hydroxy aryl group installed (**26**), we now tested the attempted transesterification under mild conditions as reported by Seebach and co-workers.<sup>[28]</sup> Using molecular sieves as a scavenger for methanol, we were able to obtain the pentenyl (**27**) and decanyl ester (**28**) in moderate yields of 28 % and 22 % with the desired concomitant deprotection of the hydroxyl group on position C-4 of the heterocycle, which is much more critical to carry out at a later stage of the synthesis. Deprotection of the pyridone ring and the phenol with TBAF and TFA, respectively, gave the desired products **9** and **10**.



Scheme 2: The transesterification route to pyridine alkaloid derivatives.

Due to the limitations of this route regarding general applicability and yield, we prepared derivatives with ketone side chains of different length following synthetic procedures established by Baldwin and Waldmann.<sup>[9, 10h]</sup> One of the key advantages of this route is the possibility to start from commercially available 2,4-dihydroxy pyridine (**29**) without having to form the heterocyclic ring. Following procedures reported by Baldwin *et al.*,<sup>[10h]</sup>



### 3. Results and discussion

Thirteen 4-hydroxy-2-pyridone compounds were tested for their activity on the different components of the endocannabinoid system (ECS). The molecular structure of the compounds is given in Table 2. Among them are natural products such as farinosone A, militarinone D, torrubiellone C, prebassianin B and pyridovericin, which have been produced by total synthesis.<sup>[10h-j, 12, 25]</sup> Entries 4 and 6 represent compounds that are structurally very similar, but have not been isolated from natural sources. As the side chain of 4-hydroxy-2-pyridones was shown to hardly have any influence on their neurotogenic activity in PC12 cell assays,<sup>[29]</sup> truncated structurally simplified derivatives with an ester and a ketone side-chain of different lengths were included as well.

First, we investigated the binding properties to CB<sub>1</sub> and CB<sub>2</sub> receptors.<sup>[30]</sup> As reported in Fig. 1A, HJJ-510 (**4**) and pretenellin B (**7**) showed a very weak binding to CB<sub>1</sub> defined by approximately 40 % displacement of [<sup>3</sup>H]CP55,940 at a concentration of 1  $\mu$ M. Compound **12** reached 50 % displacement of the radioligand and was assessed in a full concentration-dependent curve showing a K<sub>i</sub> value of 1.0  $\mu$ M (Fig. 1B). In contrast, the other structurally simplified pyridones did not show any significant binding to CB<sub>1</sub> and CB<sub>2</sub> receptors (< 20 %) at a concentration of 1  $\mu$ M (Fig 1A). Compound **12** did not bind to CB<sub>2</sub> receptors, thus showing a selective interaction with CB<sub>1</sub> receptors.

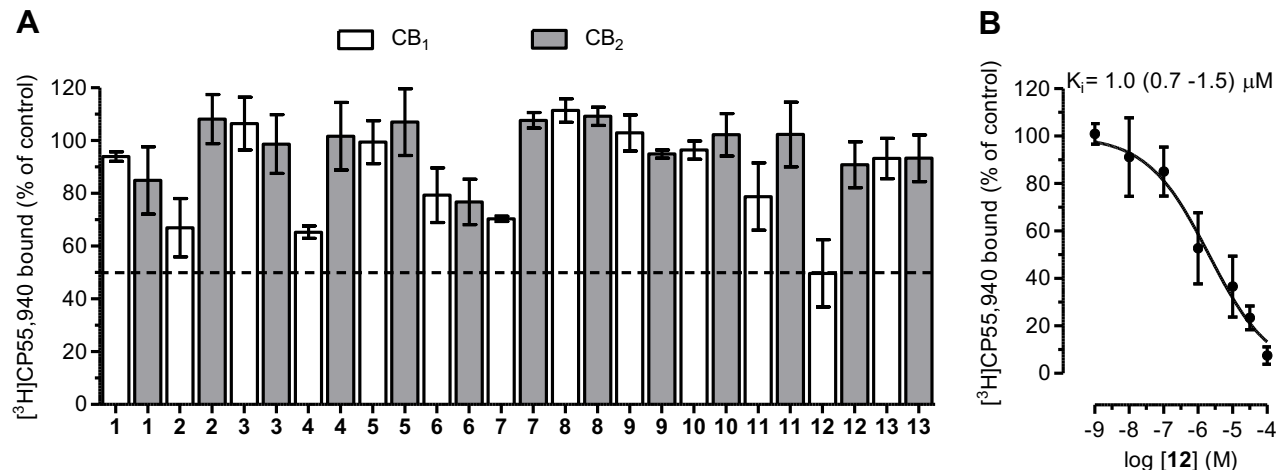


Fig. 1. **A:** CB<sub>1</sub> and CB<sub>2</sub> receptors binding properties of pyridone alkaloids tested at the screening concentration of 1  $\mu$ M. **B:** Concentration-dependent binding curve for compound **12** to CB<sub>1</sub> receptors.

We then investigated the effects of these compounds on 2-OG hydrolysis by monoacylglycerol lipase (MAGL),  $\alpha,\beta$ -hydrolase domain-6 (ABHD6) and -12 (ABHD12), as well as AEA hydrolysis by fatty acid amide hydrolase (FAAH). As shown in Fig. 2A, none of the tested compounds showed significant inhibition of MAGL activity at the concentration of 10  $\mu$ M. Similarly, our compound library did not inhibit ABHD6 and ABDH12, as compared to the positive control compounds WWL70 (**17**) and THL (**18**) used for the ABHD6 and ABHD12 assay, respectively (data not shown).<sup>[20]</sup> In contrast, militarinone D (**2**), **10** and **12** inhibited FAAH activity by 40–50 % at 10  $\mu$ M, while compound **11** inhibited AEA hydrolysis by 80 % compared to vehicle. In further concentration-dependent experiments, **11** showed an IC<sub>50</sub> value of 4.6  $\mu$ M (Fig. 2B). Interestingly, this compound did not show any significant inhibition of MAGL, thus indicating a selectivity towards FAAH inhibition.

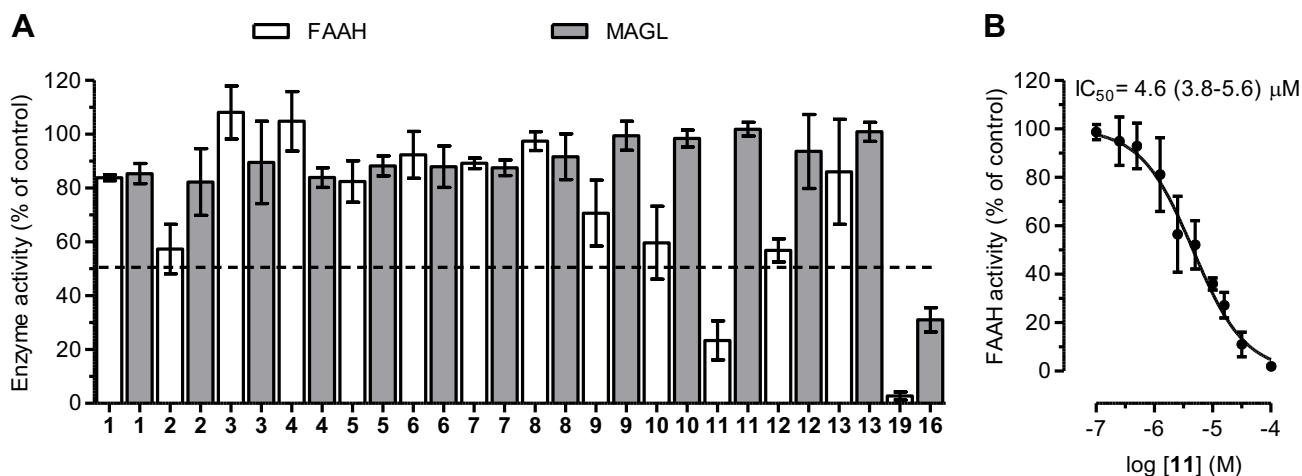


Fig. 2. **A:** FAAH and MAGL activity upon treatment with the seven natural pyridone alkaloids and five simplified analogs tested at the screening concentration of 10  $\mu$ M. URB597 (**19**) (1  $\mu$ M)<sup>[21]</sup> and JZL184 (**16**)<sup>[19]</sup> (1  $\mu$ M) were used as positive control for FAAH and MAGL inhibition, respectively. **B:** Concentration-dependent inhibition of FAAH activity induced by **11** in U937 cell homogenate.

Alternatively, AEA and 2-AG can undergo oxidative metabolism mediated by COX-2 activity.<sup>[31]</sup> This degradation pathway generates prostaglandin-ethanolamines and prostaglandin-glycerol esters, which were shown to possess bioactivity unrelated to the interaction with cannabinoid receptors and the classic prostaglandin receptors. In the last years, it has been observed that several compounds can act as specific inhibitors by blocking the COX-2-mediated oxygenation of AEA and 2-AG without affecting arachidonic acid oxidation.<sup>[32]</sup> Here, we tested the pyridone alkaloids for the inhibition of COX-2-mediated oxidation of the classic substrate arachidonic acid or the endocannabinoid 2-AG. As shown in Fig. 3, some of the compounds showed partial inhibition of COX-2 (ranging from 20 % to 50 %) at a screening concentration of 10  $\mu$ M, which was not selective for either of the substrates.

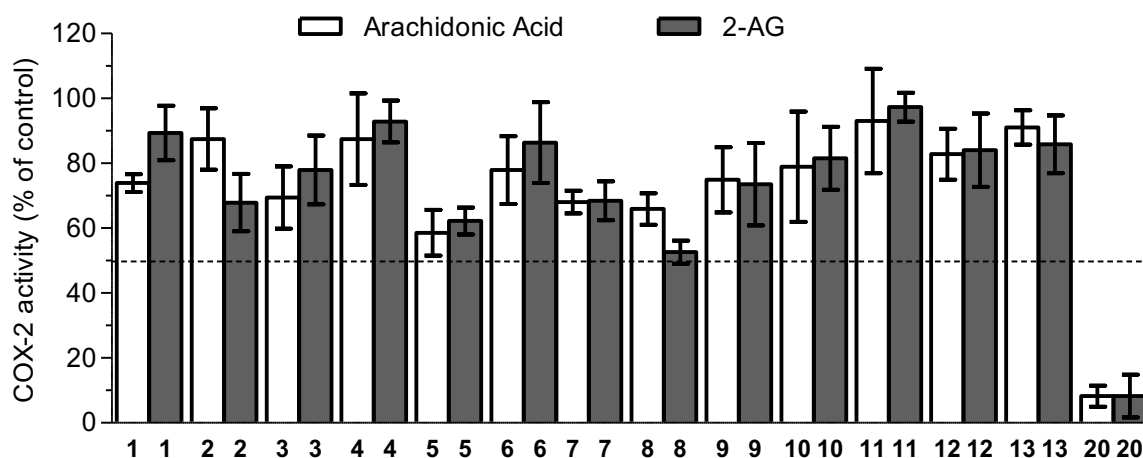


Fig. 3. COX-2 activity upon treatment with the natural pyridone alkaloids and derivatives at a screening concentration of 10  $\mu$ M using either arachidonic acid or 2-AG as substrate. DuP697 (1  $\mu$ M)<sup>[22]</sup> (**20**) was used as a positive control.

Finally, all compounds were screened on AEA uptake using U937 cells as previously described. At a concentration of 10  $\mu$ M, farinosone A (**1**) induced the most potent inhibition of AEA uptake (Fig. 4A) and in further concentration-dependent experiments, it showed an IC<sub>50</sub> value of 1.2  $\mu$ M (Fig. 4B), being in range with the potency reported for the positive controls UCM707<sup>[18]</sup> and OMDM-2.<sup>[23, 33]</sup> Militarionone D (**2**), SF-182 (**6**) and pretenellin B (**7**) only partially inhibited AEA uptake at 10  $\mu$ M, while torrubiellone C (**3**), HJJ-510 (**4**), prebassianin B (**5**) and pyridovericin (**8**) were inactive (Fig. 4A). This data indicates that,

in comparison to farinosone A (**1**), shorter side chains in combination with a lower degree of unsaturation (**4** and **5**) and in the presence of a hydroxyl group (**3** and **8**) lead to impaired binding capacity.

The synthetic analogs of the natural pyridone alkaloids (**9–13**) were also tested under the same conditions. At a concentration of 10  $\mu$ M, **10**, **11** and **12** induced maximal inhibition of AEA uptake in the same range as the positive controls (Fig. 4A). In further concentration-dependent experiments, these compounds showed IC<sub>50</sub> values in the submicromolar range (0.67–0.78  $\mu$ M) (Fig 4B). In contrast, compounds **9** and **13** were inactive (Fig. 4A). This indicates that fully saturated unbranched side chains in the range of C<sub>8</sub>–C<sub>12</sub> (**10–12**) represent the optimal length for inhibition of AEA uptake, as compared to longer (C<sub>20</sub>, **13**) and shorter (C<sub>4</sub>, **9**) chains. When comparing natural pyridone alkaloids with their synthetic analogs, the data indicates that branched side chains (**1–8**) are less favourable for AEA uptake inhibition as compared to linear alkyl side chains (**10–12**).

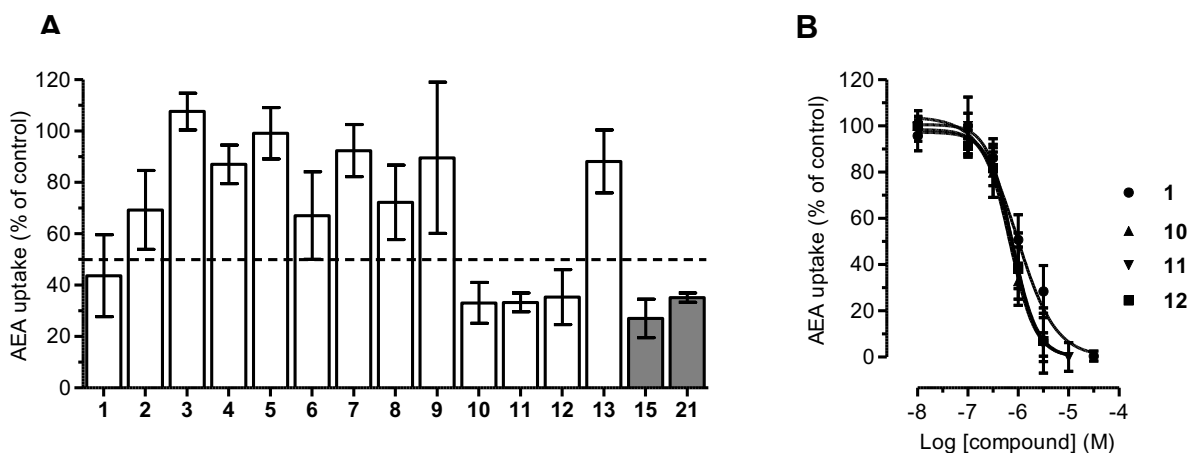


Fig. 4. **A:** Inhibition of AEA uptake into U937 cells induced by natural pyridone alkaloids and their analogs at a screening concentration of 10  $\mu$ M. **B:** The most potent inhibitors were tested in concentration-dependent experiments. UCM707 (**15**)<sup>[18]</sup> and OMDM-2 (**21**)<sup>[23]</sup> (both at 10  $\mu$ M) were used as positive controls. Data in graph **B** have been normalized.

AEA hydrolysis and uptake are coupled processes as the FAAH-dependent AEA concentration gradient needs to be maintained. This gradient is the driving force for AEA's energy-independent uptake.<sup>[16b, 34]</sup> In cellular systems, FAAH inhibitors can indirectly

inhibit AEA uptake by affecting this concentration gradient,<sup>[34-35]</sup> and a correlation between IC<sub>50</sub> values for FAAH and AEA uptake inhibition was reported.<sup>[36]</sup> Importantly, farinosone A (**1**) and the synthetic analogs **10–12** showed IC<sub>50</sub> values >10  $\mu$ M for FAAH. These compounds also showed IC<sub>50</sub> values >10  $\mu$ M for MAGL and COX-2. Thus, **1** and **10–12** are selective inhibitors of AEA uptake (Table 3). Among them, only **12** showed weak binding to the CB<sub>1</sub> receptor ( $K_i$  = 1.0  $\mu$ M), while the other compounds did not significantly bind to CB<sub>1</sub> or CB<sub>2</sub> (0–30% binding at 1  $\mu$ M).

Table 3. Summary of the  $K_i$  and IC<sub>50</sub> values of natural pyridone alkaloids **1** and their analogs **10–13** on the different components of the ECS.

Compound	$K_i$ value (CB <sub>1</sub> and CB <sub>2</sub> ) or IC <sub>50</sub> values (mean $\pm$ SD, $\mu$ M)					
	CB <sub>1</sub>	CB <sub>2</sub>	FAAH	MAGL	AEA uptake	COX-2
<b>1</b>	>1	>1	>10	>10	1.2 $\pm$ 0.6	>10
<b>10</b>	>1	>1	>10	>10	0.6 $\pm$ 0.4	>10
<b>11</b>	>1	>1	>10	>10	0.7 $\pm$ 0.4	>10
<b>12</b>	1.0 $\pm$ 0.5	>1	4.6 $\pm$ 0.7	>10	0.8 $\pm$ 0.3	>10
<b>13</b>	>1	>1	>10	>10	>10	>10

#### 4. Conclusion

In conclusion, we describe that the natural products **1**, **2**, **6** and **8** as well as synthetic pyridone alkaloids **10–12** act as AEA uptake inhibitors. Their IC<sub>50</sub> values lie in the micromolar (**1**, **2**, **6**, **8**) and in submicromolar (**10–12**) range and a selectivity >10 fold over the main components of the ECS, including FAAH. In agreement with other studies on different scaffolds, our results indicate that small modifications of the pyridone alkaloid side chain in terms of length, branching, degree of unsaturation and the presence of hydrophilic substituents can lead to a significant loss of potency for AEA uptake inhibition.<sup>[34-36]</sup>



## 5. Experimental section

### 5.1 Chemistry

The total syntheses of natural products **1–8** have been published by one of our groups and the substance samples used in this work have been produced by these procedures.<sup>[10i, 12, 25]</sup> Derivatives **9–13** are novel compounds that have not been reported elsewhere. Esters **9** and **10** are produced by transesterification of the respective methyl ester derivatives. The preparation of ketones **11–13** has been carried out in close analogy to previously reported synthetic work by the groups of Baldwin and Waldmann.<sup>[9, 10h]</sup> All synthetic procedures as well as analytical data of all intermediates can be found in the Supporting Information. Chemicals and solvents used for reactions were supplied by Sigma-Aldrich, Alfa Aesar, Fluka, Fluorochem, Maybridge, Acros, and Merck, and were used without further purification. Distilled technical grade solvents were used for column chromatography and work-up. Solvents were removed in vacuo on a rotary evaporator with 40 °C water bath temperature. Unless otherwise stated, reactions were carried out under argon atmosphere and reactions involving air-sensitive reagents were carried out using solvents degassed by freeze-pump-thaw cycling. Deuterated solvents used for NMR spectroscopy were supplied by Cambridge Isotope Laboratories. All reported yields are isolated yields, unless otherwise indicated. Flash chromatography was performed by dry-pack method using silica gel with particles size between 0.040 and 0.063 mm as stationary phase according to the procedure developed by Mitra and coworkers.<sup>[37]</sup> Thin layer chromatography was performed on silica gel-coated glass plates (Merck 60 F<sub>254</sub>, thickness: 210–270 µm), spots were observed under UV light ( $\lambda = 254$  nm) and plates were stained with potassium permanganate solution. Preparative HPLC purifications were carried out on a Dionex Chromatography System (Interface Chromeleon, ASI 100 automatic sample injector, PDA 100 (USB) PD detector, pump P680); flow rate: 20 mL min<sup>-1</sup>; column: Phenomenex Synergi Hydro-RP, 10 µm, 80 Å, 250 mm x 21.2 mm); solvents: A – H<sub>2</sub>O, B – MeCN.

Method 1: from 80 % B to 100 % B over 2 minutes and maintaining 100 % B during 40 minutes. Method 2: from 50 % B to 100 % B over 25 minutes and maintained 100 % B during 15 minutes. Reversed-phase LC-MS analyses were performed on a Shimadzu LC system with a Reprospher column (Dr. Maisch, 100 Å C18-Aqua, 5 µm, 125 mm x 2 mm)

and an amaZon X Bruker mass spectrometer. The solvents used were demineralized water (milli-Q, 0.01 % formic acid, solvent A) and acetonitrile (HPLC grade, 0.01% formic acid, solvent B) at a flow rate of 0.5 mL min<sup>-1</sup>. The gradient applied was 0.0–2.0 minutes 10 % B; 2.0–12.0 minutes 10 % B to 95 % B; 12.0–15.0 minutes 95 % B; 15.0–16.0 minutes 95 % B to 10 % B; 16.0–18.0 minutes 10 % B. NMR experiments were performed on Bruker Avance III NMR spectrometers operating at 250 MHz, 400 MHz or 500 MHz proton frequency. Chemical shifts  $\delta$  are reported in ppm and were determined by reference to the residual <sup>1</sup>H solvent peaks (chloroform: 7.26 ppm (<sup>1</sup>H) and 77.16 (<sup>13</sup>C); DMSO: 2.50 ppm (<sup>1</sup>H) and 39.52 ppm (<sup>13</sup>C); methanol: 3.31 ppm (<sup>1</sup>H), 49.00 ppm(<sup>13</sup>C)).<sup>[38]</sup> The assignment of signals in the <sup>1</sup>H and <sup>13</sup>C NMR spectra was achieved by interpretation of DEPT and two-dimensional NMR spectra (COSY, NOESY, HMBC and HSQC). Processing, analyses and interpretations of NMR spectra were performed with MestReNova (version: 10.0.1-14719). Infrared spectra were recorded using a Varian 800 FT-IR ATR Spectrometer. The absorptions were reported in cm<sup>-1</sup>, both the intensity and profile of signals were described as weak, medium, strong or broad. ESI-HRMS spectra. Melting points (m.p.) were determined in open capillaries using a Büchi B-545 apparatus.

**Methyl (Z)-2-cyano-3-methoxybut-2-enoate.**<sup>[26]</sup> In a one-necked 100 ml round-bottomed flask, methyl cyanoacetate (18.0 mL, 20.1 g, 0.203 mol, 1.0 equiv) and trimethyl orthoacetate (28.86 g, 0.240 mol, 1.2 equiv) were stirred at 110 °C and the methanol formed during this reaction was removed *via* a short distillation bridge. After three hours, a second portion of trimethyl orthoacetate was added (30.2 g, 0.251 mmol, 1.2 equiv) and the reaction mixture stirred at 110 °C for another four hours. The temperature was then raised to 135 °C and excess reagents distilled off the reaction mixture. Upon cooling, the mixture solidified. A mixture of water and methanol (75 mL, water/methanol 2/1) was added and the flask heated until the solid had completely dissolved. The hot solution was allowed to cool to room temperature and was stored at 8 °C to allow for crystallization of the product. Colorless crystals, **yield:** 62 %; **m.p.** = 97 °C; **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>)  $\delta$  3.99 (s, 3H), 3.78 (s, 3H), 2.62 (s, 3H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>)  $\delta$  184.6, 164.5, 115.1, 85.8, 56.9, 52.3, 15.3; **HRMS** (ESI+) exact mass calculated for [C<sub>7</sub>H<sub>10</sub>NO<sub>3</sub>]<sup>+</sup> *m/z* 156.0655 [M+H]<sup>+</sup>, found *m/z* 156.0656; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 2957, 2222, 1715, 1572, 1470, 1438, 1429, 1410, 1381, 1315, 1288, 1194, 1129, 1088, 1022, 969, 873, 788, 768, 641. **Methyl (2Z,4E)-2-cyano-5-(dimethylamino)-3-methoxypenta-2,4-dienoate (23).**<sup>[27]</sup> Methyl (Z)-

2-cyano-3-methoxybut-2-enoate (16.9 g, 0.109 mol, 1.0 equiv) was suspended in dry methanol (8 mL) under an inert atmosphere and *N,N*-dimethylformamide dimethylacetale (23.2 mL, 20.7 g, 0.174 mmol, 1.6 equiv) was added. The reaction mixture was stirred under reflux conditions for 45 min and turned dark red. Excess solvents and reagents were removed at the rotary evaporator. The red solid was dissolved in DCM and purified by filtration over a silica gel column. After evaporation of the solvent, the orange product was recrystallized from ethyl acetate/petroleum ether to give a bright yellow solid. Yellow crystals, **yield**: 74 %; **m.p.** = 129 °C; **R<sub>f</sub>** = 0.27 (50 % EtOAc in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.45 (d, *J* = 12.4 Hz, 1H), 6.09 (s, br, 1H), 3.93 (s, 3H), 3.65 (s, 3H), 3.13 (s, 3H), 2.90 (s, 3H, NCH<sub>3</sub>)<sub>2</sub>; **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 181.8 ((H<sub>3</sub>CO)C=C), 166.4 (COOCH<sub>3</sub>), 153.3 (C=C(NMe<sub>2</sub>)), 119.3 (CN), 91.4 ((Me<sub>2</sub>N)C=C), 74.9 (C=C(CN)), 62.5 (OCH<sub>3</sub>), 51.3 (COOCH<sub>3</sub>), 45.7 (N(CH<sub>3</sub>)<sub>2</sub>), 37.5 (N(CH<sub>3</sub>)<sub>2</sub>); **HRMS** (ESI+) exact mass calculated for [C<sub>10</sub>H<sub>15</sub>N<sub>2</sub>O<sub>3</sub>]<sup>+</sup> *m/z* 211.1077 [M+H]<sup>+</sup>, found *m/z* 211.1074; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 2927, 2917, 2187 (CN), 1690, 1612, 1525, 1459, 1432, 1389, 1359, 1315, 1265, 1225, 1205, 1185, 1133, 1052, 1000, 982, 891, 871, 823, 794, 769, 726, 643. **Methyl 4-methoxy-2-oxo-1,2-dihydropyridine-3-carboxylate**. Methyl 2-cyano-5-(dimethylamino)-3-methoxypenta-2,4-dienoate (15.1 g, 71.9 mmol) was dissolved in an aqueous solution of acetic acid (80 vol.-%, 50 ml) in a 250 ml round-bottomed flask and stirred under reflux conditions under inert gas for one hour. The dark red reaction mixture was diluted with water (25 ml) and solid sodium carbonate was carefully added until pH 7 was reached. The product was extracted from the aqueous phase with ethyl acetate (8 x 50 mL) and with DCM (4 x 50 mL). The combined organic phases (red) were dried over sodium sulfate. After filtration of the drying agent the solvent was removed under vacuum. Flash chromatography yielded the product as an orange solid (7.7 % MeOH in DCM). Orange crystals, **yield**: 40 %; **m.p.** = 202–204 °C (decomposition); **R<sub>f</sub>** = 0.29 (9.1 % MeOH in DCM); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 13.47 (s, 1H), 7.50 (d, *J* = 7.4 Hz, 1H), 6.15 (d, *J* = 7.5 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 167.5, 165.5, 163.8, 138.3, 108.4, 95.0, 56.7, 52.6; **HRMS** (ESI+) exact mass calculated for [C<sub>8</sub>H<sub>9</sub>NO<sub>4</sub>Na]<sup>+</sup> *m/z* 206.0424 [M+Na]<sup>+</sup>, found *m/z* 206.0425; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 2952, 2839, 2360, 1733, 1632, 1610, 1552, 1477, 1429, 1327, 1272, 1241, 1181, 1124, 1094, 1073, 966, 952, 919, 836, 786, 770, 674, 629. **Methyl 5-bromo-4-methoxy-2-oxo-1,2-dihydropyridine-3-carboxylate (24)**. In a 100 ml round-bottomed flask, methyl 4-

methoxy-2-oxo-1,2-dihydropyridine-3-carboxylate (5.73 g, 31.3 mmol, 1.0 equiv) was suspended in acetonitrile (50 ml). NBS (5.57 g, 13.1 mmol, 1.0 equiv; freshly crystallized from water and dried under high vacuum) and ammonium nitrate (0.25 mg, 1.37 mmol, 0.1 equiv) were added. The reaction mixture was heated to reflux for 45 min. The solvent was removed under vacuum. Ethyl acetate was added (80 ml), but the orange solid did not completely dissolve. Thus, the solid was filtered and dissolved in DCM. After evaporation of DCM, the pure product was obtained. The red solution of EtOAc was washed with brine (4 x 100 ml) and the combined organic phases were dried over sodium sulfate. After filtration of the drying agent, the solvent was removed under vacuum. The red solid residue partly dissolved in EtOAc and the solution overlain by toluene for recrystallization to give the product as slightly yellow crystals. The part of the red solid that was completely insoluble in EtOAc was collected by filtration and subjected to column chromatography (7.7 % MeOH in DCM). Slightly yellow crystals, **yield**: 96 %; **m.p.** = 154 °C (decomposition); **R<sub>f</sub>** = 0.39 (7.7 % MeOH in DCM); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 13.27 (s, 1H), 7.59 (s, 1H), 3.99 (s, 3H), 3.93 (s, 3H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 165.6, 163.9, 163.2, 137.2, 111.2, 96.5, 59.9, 53.2; **HRMS** (ESI+) exact mass calculated for [C<sub>8</sub>H<sub>8</sub>(<sup>79</sup>Br)NO<sub>4</sub>Na]<sup>+</sup> *m/z* 283.9529 [M+Na]<sup>+</sup>, found *m/z* 283.9528; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3005, 2951, 2900, 2809, 2774, 2686, 1728, 1618, 1475, 1464, 1448, 1432, 1391, 1317, 1266, 1246, 1212, 1099, 1070, 984, 959, 877, 860, 778, 766, 746, 692, 674. **Methyl 5-bromo-4-methoxy-2-oxo-1-{[2-(trimethylsilyl)ethoxy]methyl}-1,2-dihydropyridine-3-carboxylate (25a).**<sup>[12]</sup> To an ice-cooled solution of methyl 5-bromo-4-methoxy-2-oxo-1,2-dihydropyridine-3-carboxylate (2.989 g, 11.40 mmol, 1.0 equiv) in dry DCM (24 mL), 2-(trimethylsilyl)ethoxymethyl chloride (2.4 mL, 14 mmol, 1.2 equiv) and freshly distilled triethylamine (2.1 mL, 15 mmol, 1.3 equiv) were added. The reaction mixture was stirred at 0 °C for ten minutes and then further stirred at room temperature for four hours. The reaction mixture was diluted with DCM and washed with brine (200 mL). The aqueous phase was extracted with DCM (2 x 200 mL). The combined organic phases were dried over sodium sulfate, filtered and dried *in vacuo*. The product mixture was subjected to column chromatography (gradient: Et<sub>2</sub>O in pentane 25 % to 33 %). Colorless waxy solid, **yield**: 40 % (O-SEM product: 33 %); **m.p.** = 60–62 °C; **R<sub>f</sub>**: 0.39 (6.3 % MeOH in DCM); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.61 (s, 1H), 5.26 (s, 2H), 3.99 (s, 3H), 3.92 (s, 3H), 3.62 (m, 2H), 0.98–0.88 (m, 2H), 0.00 (s, 9H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 165.7, 161.5, 160.9,

137.1, 111.0, 95.9, 76.4, 67.8, 59.6, 53.2, 18.1, -1.3; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{14}\text{H}_{22}\text{BrNNaO}_5\text{Si}]^+$   $m/z$  414.0343  $[\text{M}(^{79}\text{Br})+\text{Na}]^+$ , found  $m/z$  414.0340,  $[\text{M}(^{79}\text{Br})+\text{Na}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 2952, 2895, 1733, 1645, 1605, 1544, 1462, 1388, 1323, 1249, 1220, 1141, 1082, 992, 916, 835, 757, 689, 623. **Methyl 4-methoxy-5-{4-[(4-methoxybenzyl)-oxy]phenyl}-2-oxo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1,2-dihydropyridine-3-carboxylate (26).**<sup>[12]</sup> A 25 mL Schlenk tube was loaded with ethyl 5-bromo-4-methoxy-2-oxo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1,2-dihydropyridine-3-carboxylate (1.764 g, 4.496 mmol, 1.0 equiv),  $\text{K}_2\text{CO}_3$  (1.865 g, 13.494 mmol, 3.0 equiv),  $[\text{Pd}(\text{PPh}_3)_4]$  (520 mg, 0.450 mmol, 0.1 equiv) and {4-[(4-methoxybenzyl)oxy]phenyl}boronic acid (1.743 g, 6.754 mmol, 1.5 equiv). The previously degassed solvent mixture (DME/water/DMF 9/1/0.5; 9 mL) was added. The suspension was frozen in liquid nitrogen and was degassed in three freeze-pump-thaw-cycles. The reaction was then stirred for 16 hours at 60 °C. The reaction mixture was diluted with EtOAc (100 mL), washed with a saturated aqueous solution of ammonium chloride (1 x 100 mL) and with brine (2 x 100 mL). The organic phase was dried over sodium sulfate, filtered and the solvents were removed *in vacuo*. The crude product was purified by column chromatography (25 % pentane in diethyl ether + 0.1 %  $\text{Et}_3\text{N}$ ). Colorless solid, **yield**: 92 %;  **$R_f$**  = 0.20 (25 % pentane in diethyl ether + 0.1 %  $\text{Et}_3\text{N}$ );  **$^1\text{H}$  NMR** (500 MHz,  $\text{CDCl}_3$ )  $\delta$  7.37 (m, 2H), 7.34 (s, 1H), 7.33 (m, 2H), 6.99 (m, 2H), 6.93 (m, 2H), 5.33 (s, 2H), 5.01 (s, 2H), 3.94 (s, 3H), 3.82 (s, 3H), 3.73 (s, 3H), 3.67 (m, 2H), 0.95 (m, 2H), 0.00 (s, 9H);  **$^{13}\text{C}$  NMR** (126 MHz,  $\text{CDCl}_3$ )  $\delta$  166.6, 164.0, 161.2, 159.7, 158.7, 136.1, 130.3, 129.4, 128.8, 126.2, 116.7, 115.0, 114.2, 111.6, 76.3, 70.0, 67.6, 59.4, 55.4, 53.0, 18.2, -1.2; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{28}\text{H}_{36}\text{NO}_7\text{Si}]^+$   $m/z$  526.2256  $[\text{M}+\text{H}]^+$ , found  $m/z$  526.2256,  $[\text{M}+\text{H}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 2955, 1733, 1612, 1587, 1561, 1518, 1462, 1448, 1401, 1389, 1366, 1330, 1308, 1266, 1247, 1208, 1166, 1079, 1056, 1036, 1007, 96, 976, 958, 904, 856, 832, 815, 753, 692, 668, 637.

#### *General Procedure for the Pyridone Methyl Ester Transesterification*<sup>[28]</sup>

A two-necked flask equipped with an adapter piece featuring a Por2 frit was filled with molecular sieves (4 Å), thoroughly flame-dried and flushed with argon. The flask was charged with 4-methoxy-5-{4-[(4-methoxybenzyl)oxy]phenyl}-2-oxo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1,2-dihydro pyridine-3-carboxylate (1.0 equiv) and LiBr (5–6 equiv). Then, the flask was evacuated under high vacuum and flushed with argon. The solvent mixture (25 % DCM in THF, 0.1 M with respect to the pyridone methyl ester derivative) was added

and the solution was stirred for 15 min. The alcohol (1.8–2.5 equiv) and DBU (0.5 equiv) were added over a septum via a syringe and the reaction mixture was heated to reflux under an atmosphere of argon for eight to nine hours. DCM and a saturated aqueous solution of ammonium chloride were added to the reaction mixture. The phases were separated and the aqueous phase was extracted with DCM (3 x 50 mL). The combined organic phases were dried over sodium sulfate. After filtration of the drying agent, the solvents were removed *in vacuo*. Preparative HPLC (method 1) was carried out to obtain the corresponding pyridone ester derivative.

**Pent-4-en-1-yl 4-hydroxy-5-{4-[(4-methoxybenzyl)oxy]phenyl}-2-oxo-1-{[2-(trimethyl silyl)ethoxy]methyl}-1,2-dihydropyridine-3-carboxylate (27).** Colorless solid, **yield:** 22%, **m.p.** = 83 °C;  $R_f$  = 0.52 (25 % pentane in Et<sub>2</sub>O); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  14.12, 7.54 (s, 1H), 7.39 (m, 2H), 7.36 (m, 2H), 7.01 (m, 2H), 6.92 (m, 2H), 5.88 (ddt,  $J$  = 17.0, 10.2, 6.6 Hz, 1H), 5.29 (s, 2H), 5.08 (dd,  $J$  = 17.0, 1.6 Hz, 1H), 5.02 (s, 2H), 5.00 (m, 1H), 4.38 (t,  $J$  = 6.7 Hz, 2H), 3.81 (s, 3H), 3.60 (m, 2H), 2.20 (m, 2H), 1.89 (m, 2H), 0.93 (m, 2H), 0.00 (s, 9H); **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>)  $\delta$  173.8, 173.0, 159.8, 159.7, 158.8, 140.1, 137.5, 130.4, 129.4, 128.9, 125.4, 115.5, 115.0, 114.2, 114.1, 98.6, 76.4, 70.0, 67.5, 65.6, 55.5, 30.1, 27.7, 18.2, -1.2; **HRMS** (ESI+) exact mass calculated for [C<sub>31</sub>H<sub>40</sub>NO<sub>7</sub>Si]<sup>+</sup>  $m/z$  566.2569 [M+H]<sup>+</sup>, found  $m/z$  566.2563, [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3075, 2998, 2952, 2901, 2880, 1731, 1665, 1612, 1515, 1468, 1446, 1406, 1347, 1323, 1300, 1283, 1241, 1218, 1178, 1140, 1080, 1032, 1007, 974, 944, 920, 833, 823, 762, 744, 708, 693, 650, 641, 611. **Decyl 4-hydroxy-5-{4-[(4-methoxybenzyl)oxy]phenyl}-2-oxo-1-{[2-(trimethylsilyl)ethoxy] methyl}-1,2-dihydropyridine-3-carboxylate (28).** Colorless solid, **yield:** 28 %;  $R_f$  = 0.70 (4.8 % MeOH in DCM); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>)  $\delta$  14.18 (s, 1H), 7.54 (s, 1H), 7.37 (m, 2H), 7.36 (dd,  $J$  = 2.2, 1.5 Hz, 2H), 7.08–6.96 (m, 2H), 6.91 (m, 2H), 5.34 (s, 2H), 5.02 (s, 2H), 4.40 (t,  $J$  = 7.1 Hz, 2H), 3.82 (s, 3H), 3.69–3.64 (m, 2H), 1.85 (m, 2H), 1.47–1.40 (m, 2H), 1.38–1.23 (m, 12H), 1.00–0.93 (m, 2H), 0.88 (t,  $J$  = 7.0 Hz, 3H), 0.01 (s, 9H); **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>)  $\delta$  173.8, 173.0, 159.7, 159.6, 158.7, 140.0, 130.3, 129.3, 128.9, 125.4, 114.9, 114.1, 114.0, 98.5, 76.3, 70.0, 67.4, 66.3, 55.4, 32.0, 29.7, 29.6, 29.4, 29.4, 28.7, 25.9, 22.8, 18.2, 14.2, -1.3; **HRMS** (ESI+) exact mass calculated for [C<sub>36</sub>H<sub>52</sub>NO<sub>7</sub>Si]<sup>+</sup>  $m/z$  638.3508 [M+H]<sup>+</sup>, found  $m/z$  638.3507, [M+H]<sup>+</sup>.

*General Procedure for N-SEM Deprotection of Pyridone Derivatives*<sup>[12]</sup>

To a solution of the *N*-SEM protected pyridone ester in THF (dry, 0.1 M) was added a solution of TBAF in THF (dry, 1 M, 2.2 equiv) *via* syringe. The reaction mixture was heated to 65 °C for 48 hours. The reaction mixture was concentrated *in vacuo*, diluted with DCM and poured on brine/DCM. The phases were separated and the aqueous phase was extracted with DCM. The combined organic phases were dried over sodium sulfate. After filtration of the drying agent, the solvent was evaporated *in vacuo*. The crude product was suspended in cold MeOH (–20 °C). The solvent was removed *via* a teflon cannula with a filter paper attached to it by teflon tape, and rinsed with cold MeOH to obtain the pure compound.

**Pent-4-en-1-yl 4-hydroxy-5-{4-[(4-methoxybenzyl)oxy]phenyl}-2-oxo-1,2-dihydropyridine-3-carboxylate.** Colorless solid, **yield:** 81%; **m.p.** = 192 °C; **R<sub>f</sub>** = 0.18 (25 % pentane in Et<sub>2</sub>O); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 14.01 (s, 1H), 12.64 (s, 1H), 7.47 (s, 1H), 7.41–7.32 (m, 4H), 7.04 (m, 2H), 6.92 (m, 2H), 5.85 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 5.16–4.96 (m, 4H), 4.43 (t, *J* = 6.9 Hz, 2H), 3.82 (s, 3H), 2.15 (m, 2H), 1.93 (m, 2H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>): δ 174.3, 172.5, 162.8, 159.6, 158.7, 138.8, 137.5, 130.3, 129.4, 129.0, 125.3, 115.6, 115.0, 114.4, 114.2, 98.8, 65.6, 55.4, 30.1, 27.8; the <sup>13</sup>C signal of C<sup>12</sup>H<sub>2</sub> was not observed; **HRMS** (ESI+) exact mass calculated for [C<sub>25</sub>H<sub>26</sub>NO<sub>6</sub>]<sup>+</sup> *m/z* 436.1755 [M+H]<sup>+</sup>, found *m/z* 436.1748 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>–1</sup>): 2975, 2911, 2845, 2758, 1647, 1549, 1514, 1487, 1451, 1412, 1382, 1351, 1307, 1277, 1236, 1206, 1178, 1080, 1022, 971, 889, 826, 764, 729, 610. **Decyl 4-hydroxy-5-{4-[(4-methoxybenzyl)oxy]phenyl}-2-oxo-1,2-dihydropyridine-3-carboxylate.** Colorless solid, **yield:** 53 %; **R<sub>f</sub>** = 0.17 (4.8 % MeOH in DCM); **<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 14.07 (s, 1H), 12.21 (s, 1H), 7.45 (s, 1H), 7.41–7.37 (m, 4H), 7.35 (m, 2H), 5.02 (s, 2H), 4.41 (t, *J* = 7.1 Hz, 2H), 3.83 (s, 3H), 1.84 (m, 2H), 1.45 (m, 2H), 1.37–1.13 (m, 12H), 0.86 (t, *J* = 7.0 Hz, 3H); **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 174.3, 172.6, 162.6, 159.7, 158.8, 138.6, 130.4, 129.4, 129.0, 125.3, 115.0, 114.4, 114.2, 98.9, 70.0, 66.4, 55.5, 32.0, 29.7, 29.7, 29.4, 29.4, 28.7, 26.0, 22.8, 14.3; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>–1</sup>): 2921, 2834, 1725, 1680, 1634, 1552, 1515, 1466, 1441, 1410, 1346, 1278, 1243, 1178, 1078, 1037, 956, 873, 829, 742, 652, 612.

#### *General Procedure for PMB Deprotection of Pyridone Ester Derivatives<sup>[12]</sup>*

To an ice-cooled solution of PMB-protected pyridone ester derivative in DCM (0.2 M) was added a solution of TFA in DCM (2.5 weight-%, 15 equiv). The reaction mixture was stirred with cooling for 30 minutes and then allowed to warm to room temperature (45–60

minutes). The reaction mixture was diluted with DCM and poured on brine. The phases were separated and the aqueous phase was extracted with DCM. The organic phases were dried over sodium sulfate. After filtration of the drying agent, the solvent was removed *in vacuo*. The resulting colorless solid was purified *via* flash chromatography (6.3 % MeOH in DCM).

**Pent-4-en-1-yl 4-hydroxy-5-(4-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (9).** Colorless solid, **yield:** 48 %; **m.p.** = 225–230 °C (decomposition);  $R_f$  = 0.15 (6.3 % MeOH in DCM);  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.47 (s, 1H), 7.32–7.23 (m, 2H), 6.81 (m, 2H), 5.87 (ddt,  $J$  = 17.0, 10.2, 6.7 Hz, 1H), 5.03 (m, 2H), 4.39 (t,  $J$  = 6.7 Hz, 2H), 2.25 (m, 2H), 1.89 (m, 2H);  $^{13}\text{C NMR}$  (101 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.4, 173.5, 162.8, 158.3, 139.8, 138.9, 131.4, 125.3, 116.1, 115.7, 101.4, 66.3, 31.1, 28.8; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{17}\text{H}_{18}\text{NO}_5]^+$   $m/z$  316.1179  $[\text{M}+\text{H}]^+$ , found  $m/z$  316.1176,  $[\text{M}+\text{H}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 3214, 2987–2757, 1650, 1540, 1513, 1484, 1443, 1418, 1343, 1303, 1276, 1249, 1202, 1175, 1110, 1081, 994, 950, 910, 870, 836, 724, 678. **Decyl 4-hydroxy-5-(4-hydroxyphenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (10).** Colorless solid, **yield:** 62 %; **m.p.** = 190 °C;  $R_f$  = 0.10 (6.3 % MeOH in DCM);  $^1\text{H NMR}$  (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.45 (s, 1H), 7.28 (m, 2H), 6.81 (m, 2H), 4.37 (t,  $J$  = 6.8 Hz, 2H), 1.79 (m, 2H), 1.55 (m, 2H), 1.37 (m, 12H), 0.90 (m, 3H);  $^{13}\text{C NMR}$  (101 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  175.5, 173.5, 162.8, 158.3, 139.7, 131.4, 125.4, 116.1, 99.9, 66.9, 33.1, 30.7, 30.7, 30.5, 30.4, 29.6, 27.0, 23.7, 14.4; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{22}\text{H}_{30}\text{NO}_5]^+$   $m/z$  388.2118  $[\text{M}+\text{H}]^+$ , found  $m/z$  388.2119,  $[\text{M}+\text{H}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 3271, 2923, 2852, 2439, 2287, 1640, 1543, 1513, 1470, 1423, 1343, 1310, 1244, 1192, 1106, 1076, 1002, 896, 837, 720, 653.

#### *General Procedure for Regioselective Lithiation and Alkylation of 3,5-Dibromo-2,4-dimethoxypyridine<sup>[9]</sup>*

In a flame-dried two-necked flask, 3,5-dibromo-2,4-dimethoxypyridine (1.0 equiv) was dissolved in dry THF (0.1 M with respect to the 3,5-dibromo-2,4-dimethoxypyridine). This solution was then cooled down to –78 °C and a previously titrated *s*-BuLi solution (1.35 M in cyclohexane, 1.0–1.2 equiv) was added dropwise over 10–15 minutes. The mixture was stirred for one hour at –78 °C. A solution of the aldehyde in dry THF (1.2 M, 1.0–2.5 equiv) was slowly added. The mixture was stirred for another hour at –78 °C. The mixture was then allowed to warm to room temperature with continued stirring (14–16 hours). The



mixture was quenched with water (1/3 of the volume of THF). A saturated aqueous ammonium chloride solution (1/3 of the volume of THF) and ethyl acetate (two times the volume of THF) were added. The phases were separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and dried over magnesium sulfate. After filtration of the drying agent, the solvents were removed *in vacuo*. The crude product was purified by flash chromatography (7 % ethyl acetate in pentane).

**1-(5-Bromo-2,4-dimethoxypyridin-3-yl)octan-1-ol (31).** Colorless liquid, **yield:** 69%; **R<sub>f</sub>** = 0.15 (7% ethyl acetate in pentane); **<sup>1</sup>H NMR** (250 MHz, CDCl<sub>3</sub>) δ 8.12 (s, 1H), 4.93 (m, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 3.21 (d, *J* = 11.5 Hz, 1H), 1.94–1.81 (m, 1H), 1.69–1.63 (m, 1H), 1.26 (m, 10H), 0.86 (t, *J* = 6.7 Hz, 3H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 162.2, 162.0, 147.8, 121.3, 108.1, 67.9, 61.8, 54.1, 37.3, 32.0, 29.5, 29.3, 26.3, 22.8, 14.2; **HRMS** (ESI+) exact mass calculated for [C<sub>15</sub>H<sub>25</sub>O<sub>3</sub>NBr]<sup>+</sup> *m/z* 346.10123 [M(<sup>79</sup>Br)+H]<sup>+</sup>, found *m/z* 346.1012 [M(<sup>79</sup>Br)+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3568, 3446, 2925, 2855, 1729, 1564 1458, 1416, 1381, 1297, 1250, 1160, 1086, 1008, 914, 832, 794, 723, 663, 624. **1-(5-Bromo-2,4-dimethoxypyridin-3-yl)dodecan-1-ol (32).** Colorless oil, **yield:** 93 %; **R<sub>f</sub>** = 0.17 (7 % ethyl acetate in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.13 (s, 1H), 4.95–4.92 (m, 1H), 3.97 (s, 3H), 3.91 (s, 3H), 3.20 (bs, 1H), 1.89 (m, 1H), 1.70 (m, 1H), 1.48 (m, 1H), 1.32–1.21 (m, 17H), 0.87 (t, *J* = 6.8 Hz, 3H); **HRMS** (ESI+) exact mass calculated for [C<sub>19</sub>H<sub>33</sub>BrNO<sub>3</sub>]<sup>+</sup> *m/z* 402.16383 [M(<sup>79</sup>Br)+H]<sup>+</sup>, found *m/z* 402.1639 [M(<sup>79</sup>Br)+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3572, 3436, 2923, 2853, 1729, 1564 1459, 1416, 1381, 1298, 1250, 1162, 1089, 1009, 914, 826, 794, 664, 630. **1-(5-Bromo-2,4-dimethoxypyridin-3-yl)octadecan-1-ol (33).** Colorless solid, **yield:** 77 %; **m.p.** = 52 °C; **R<sub>f</sub>** = 0.24 (7 % ethyl acetate in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.11 (s, 1H), 4.995–4.89 (m, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.19 (d, *J* = 11.2 Hz, 1H), 1.94–1.81 (m, 1H), 1.70–1.61 (m, 1H), 1.33–1.17 (m, 30H), 0.85 (t, *J* = 6.8 Hz, 3H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 162.1, 162.0, 147.7, 121.3, 108.1, 67.8, 61.7, 54.1, 37.3, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 29.5, 26.3, 22.8, 14.2; **HRMS** (ESI+) exact mass calculated for [C<sub>25</sub>H<sub>45</sub>BrNO<sub>3</sub>]<sup>+</sup> *m/z* 486.25773 [M(<sup>79</sup>Br)+H]<sup>+</sup>, found *m/z* 486.2576 [M(<sup>79</sup>Br)+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3440, 3297, 2919, 2851, 1565 1461, 1416, 1381, 1297, 1251, 1119, 1089, 1012, 936, 910, 844, 794, 665, 628.

*General Procedure for Suzuki Cross-Coupling Reactions*<sup>[9]</sup>

A flame-dried Schlenk tube was charged with 5-bromo-2,4-dimethoxypyridine (1.0 equiv), 4-[(*tert*-butyldimethylsilyl)oxy]phenyl boronic acid (1.8–2.0 equiv) and [Pd(PPh<sub>3</sub>)<sub>4</sub>] (8 mol%). The flask was evacuated and flushed with argon. A degassed mixture of toluene/ethanol (20 % ethanol in toluene, 0.05 M with respect to the 5-bromo-2,4-dimethoxypyridine) and a degassed aqueous solution of sodium carbonate (2 M, 70 Vol-% of the organic solvent) were added. The reaction mixture was heated to reflux (85 °C) for 14–20 hours. Ethyl acetate was added and the phases were separated. The organic phase was washed with water and brine, and dried over anhydrous magnesium sulfate. After filtration of the drying agent, the solvent was removed *in vacuo*. The crude product was purified by column chromatography (7 % ethyl acetate in pentane).

**1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}octan-1-ol (34).** Colorless liquid, **yield:** 94 %; **R<sub>f</sub>** = 0.07 (7 % ethyl acetate in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.96 (s, 1H), 7.38–7.29 (m, 2H), 6.94–6.86 (m, 2H), 5.03 (ddd, *J* = 11.6, 8.4, 5.8 Hz, 1H), 4.01 (s, 3H), 3.42 (d, *J* = 11.6 Hz, 1H), 3.39 (s, 3H), 1.97–1.84 (m, 1H), 1.78–1.68 (m, 1H), 1.59–1.46 (m, 1H), 1.35–1.24 (m, 8H), 1.00 (s, 9H), 0.88 (t, *J* = 6.8 Hz, 3H), 0.23 (s, 6H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 163.2, 161.7, 155.4, 146.8, 130.0, 128.5, 124.9, 120.4, 118.6, 67.8, 60.8, 53.8, 37.8, 32.0, 29.6, 29.4, 26.4, 25.8, 22.8, 18.4, 14.3, -4.2; **HRMS** (ESI+) exact mass calculated for C<sub>27</sub>H<sub>44</sub>NO<sub>4</sub>Si *m/z* 474.30341 [M+H]<sup>+</sup>, found *m/z* 474.3040 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3568, 3462, 2929, 2857, 1589, 1513, 1467, 1413, 1377, 1255, 1171, 1136 1072, 1012, 911, 840, 806, 781, 657, 624. **1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}dodecan-1-ol (35).** Colorless liquid, **yield:** 94 %; **R<sub>f</sub>** = 0.09 (7 % ethyl acetate in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 7.96 (s, 1H), 7.38–7.30 (m, 2H), 6.96–6.84 (m, 2H), 5.03 (ddd, *J* = 11.6, 8.3, 5.8 Hz, 1H), 4.01 (s, 3H), 3.42 (d, *J* = 11.6 Hz, 1H), 3.39 (s, 3H), 1.96–1.85 (m, 1H), 1.77–1.66 (m, 1H), 1.53 (m, 1H), 1.38–1.20 (m, 17H), 1.00 (s, 9H), 0.87 (t, *J* = 6.7 Hz, 3H), 0.23 (s, 6H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 163.2, 161.7, 155.4, 146.8, 130.0, 128.5, 124.9, 120.4, 118.6, 67.8, 60.8, 53.8, 37.8, 32.1, 29.8, 29.8, 29.8 29.7, 29.5, 26.4, 25.8, 22.8, 18.4, 14.3, -4.2; **HRMS** (ESI+) exact mass calculated for [C<sub>31</sub>H<sub>52</sub>NO<sub>4</sub>Si]<sup>+</sup> *m/z* 530.36601 [M+H]<sup>+</sup>, found *m/z* 530.3666 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3566, 2927, 2852, 1590, 1514, 1468, 1423, 1377, 1258, 1171, 1136 1073, 1013, 912, 842, 782, 735, 688, 632. **1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}octadecan-1-ol (36).** Colorless solid, **yield:** 93 %, **m.p.** = 48 °C; **R<sub>f</sub>** = 0.10 (7 % ethyl acetate in pentane);

**<sup>1</sup>H NMR** (500 MHz, CDCl<sub>3</sub>) δ 7.96 (s, 1H), 7.38–7.29 (m, 2H), 6.94–6.85 (m, 2H), 5.03 (ddd, *J* = 11.6, 8.3, 5.8 Hz, 1H), 4.01 (s, 3H), 3.41 (d, *J* = 11.6 Hz, 1H), 3.39 (s, 3H), 1.95–1.86 (m, 1H), 1.75–1.68 (m, 1H), 1.56–1.47 (m, 1H), 1.35–1.23 (m, 29H), 1.00 (s, 9H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.23 (s, 6H); **<sup>13</sup>C NMR** (126 MHz, CDCl<sub>3</sub>) δ 163.2, 161.7, 155.4, 146.8, 130.0, 128.5, 124.9, 120.4, 118.6, 67.8, 60.8, 53.8, 37.9, 32.1, 29.9, 29.9, 29.8, 29.8, 29.8, 29.7, 29.5, 26.4, 25.8, 22.9, 18.4, 14.3, –4.2; **HRMS** (ESI+) exact mass calculated for C<sub>37</sub>H<sub>64</sub>NO<sub>4</sub>Si *m/z* 614.45991 [M+H]<sup>+</sup>, found *m/z* 614.4609 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>–1</sup>): 3574, 2924, 2854, 1590, 1514, 1468, 1423, 1377, 1258, 1171, 1135 1073, 1014, 913, 842, 806 782, 719, 632.

#### *General Procedure for Dess-Martin Oxidation*<sup>[9]</sup>

The racemic mixture of secondary pyridine alcohol (1.0 equiv) was dissolved in dry DCM (0.02 M) and Dess-Martin periodinane (1.5–1.8 equiv) was added portionwise. The colorless suspension was stirred at room temperature until completion of the reaction (1–2 hours). The reaction mixture was quenched with a saturated aqueous solution of sodium hydrogen carbonate and ethyl acetate. The mixture was treated with solid sodium thiosulfate (300 mg per 100 mg of Dess-Martin periodinane) and stirred until the precipitate was dissolved. The phases were separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over magnesium sulfate and the solvent was removed *in vacuo*. The product was purified by elution through a short silica gel column (7 % ethyl acetate in petroleum ether).

**1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}octan-1-one.** Colorless oil, **yield:** 92 %; ***R*<sub>f</sub>** = 0.46 (7 % ethyl acetate in petroleum ether); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.06 (s, 1H), 7.33 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 3.96 (s, 3H), 3.44 (s, 3H), 2.81 (t, *J* = 7.4 Hz, 2H), 1.70 (p, *J* = 7.4 Hz, 2H), 1.39–1.24 (m, 8H), 1.00 (s, 9H), 0.92–0.86 (m, 3H), 0.23 (s, 6H); **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>) δ 203.9, 162.7, 160.6, 155.6, 148.7, 130.1, 127.8, 124.6, 120.4, 118.2, 61.4, 54.1, 44.9, 31.9, 29.3, 29.2, 25.8, 23.7, 22.8, 18.4, 14.2, –4.2; **HRMS** (ESI+) exact mass calculated for [C<sub>27</sub>H<sub>42</sub>NO<sub>4</sub>Si]<sup>+</sup> *m/z* 472.28776 [M+H]<sup>+</sup>, found *m/z* 472.2879 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>–1</sup>): 2930, 2858, 1708, 1588, 1513, 1467, 1409, 1378, 1259, 1172, 1142, 1110 1085, 1009, 913, 841, 782, 668, 632. **1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}-dodecan-1-one.** Colorless oil, **yield:** 82 % ; ***R*<sub>f</sub>** = 0.48 (7 % ethyl acetate in pentane); **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>) δ 8.06 (s, 1H), 7.37–7.29 (m, 2H), 6.94–6.85 (m, 2H), 3.96 (s,

3H), 3.44 (s, 3H), 2.81 (t,  $J = 7.4$  Hz, 2H), 1.75–1.64 (m, 2H), 1.28 (m, 16H), 1.04–0.94 (m, 9H), 0.87 (t,  $J = 6.8$  Hz, 3H), 0.23 (s, 6H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  203.9, 162.7, 160.5, 155.6, 148.7, 130.1, 127.8, 124.6, 120.4, 118.2, 61.4, 54.0, 44.9, 32.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 25.8, 23.7, 22.8, 18.4, 14.3, –4.2; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{31}\text{H}_{50}\text{NO}_4\text{Si}]^+$   $m/z$  528.35036  $[\text{M}+\text{H}]^+$ , found  $m/z$  528.3510  $[\text{M}+\text{H}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 2928, 2856, 1709, 1588, 1516, 1468, 1409, 1378, 1317, 1260, 1172, 1090, 1010, 913, 841, 782, 688, 632. **1-{5-[4-((*tert*-Butyldimethylsilyl)oxy)phenyl]-2,4-dimethoxypyridin-3-yl}octadecan-1-one**. Colorless solid, **yield**: 94 %, **m.p.** = 42 °C;  $R_f$  = 0.51 (7 % ethyl acetate in pentane);  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.06 (s, 1H), 7.40–7.30 (m, 2H), 6.94–6.84 (m, 2H), 3.96 (s, 3H), 3.44 (s, 3H), 2.81 (t,  $J = 7.4$  Hz, 2H), 1.70 (p,  $J = 7.4$  Hz, 2H), 1.36–1.22 (m, 28H), 1.00 (s, 9H), 0.88 (t,  $J = 6.9$  Hz, 3H), 0.23 (s, 6H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  203.9, 162.7, 160.5, 155.6, 148.7, 130.1, 127.8, 124.6, 120.4, 118.2, 61.4, 54.0, 44.9, 32.1, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 25.8, 23.7, 22.8, 18.4, 14.3, –4.2; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{37}\text{H}_{62}\text{NO}_4\text{Si}]^+$   $m/z$  612.4443  $[\text{M}+\text{H}]^+$ , found  $m/z$  612.4433  $[\text{M}+\text{H}]^+$ ; **FT-IR**  $\tilde{\nu}$  (neat,  $\text{cm}^{-1}$ ): 2919, 2851, 2361, 1703, 1585, 1559, 1515, 1462, 1407, 1364, 1328, 1258, 1173, 1135, 1107, 1076, 1005, 914, 839, 806, 780, 750, 719, 722, 704, 681, 654.

#### *General Procedure for Pyridine Deprotection<sup>[9]</sup>*

The protected pyridine (1.0 equiv) was dissolved in MeCN (dry, 0.01–0.02 M). Dry aluminium chloride (25 equiv) and dry sodium iodide (20 equiv) were added. The mixture was stirred at room temperature or 45 °C for one to two hours. The reaction mixture was quenched with a few milliliters of hydrochloric acid (1 M, aq) and extracted four times with ethyl acetate. The combined organic phases were washed with an aqueous solution of sodium thiosulfate (5 weight-%), brine and dried over magnesium sulfate. The solvent was removed *in vacuo* and the crude product was purified by preparative HPLC (MeCN/water). **4-Hydroxy-5-(4-hydroxyphenyl)-3-octanoylpyridin-2(1H)-one (11)**. Colorless solid, **yield**: 68 %, **m.p.** = 154 °C;  $R_f$  = 0.29 (6.3 % methanol in DCM);  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$  11.67 (d,  $J = 6.5$  Hz, 1H), 9.48 (s, 1H), 7.56 (d,  $J = 6.5$  Hz, 1H), 7.30–7.19 (m, 2H), 6.82–6.71 (m, 2H), 3.12 (t,  $J = 7.4$  Hz, 2H), 1.58 (p,  $J = 7.0$  Hz, 2H), 1.37–1.19 (m, 8H), 0.90–0.81 (m, 3H);  $^{13}\text{C}$  NMR (101 MHz,  $\text{DMSO}-d_6$ )  $\delta$  208.1, 175.3, 161.4, 156.7, 140.7, 130.1, 123.3, 115.0, 112.2, 106.0, 41.9, 31.2, 28.7, 28.6, 23.9, 22.1, 13.9; **HRMS** (ESI+) exact mass calculated for  $[\text{C}_{19}\text{H}_{24}\text{NO}_4]^+$   $m/z$  330.1700  $[\text{M}+\text{H}]^+$ , found  $m/z$  330.1695

[M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3295, 2925, 2854, 1659, 1607, 1542, 1512, 1453, 1427, 1379, 1231, 1175, 1109, 988, 901, 833, 705, 635. **3-Dodecanoyl-4-hydroxy-5-(4-hydroxyphenyl)pyridin-2(1H)-one (12)**. Colorless solid, **yield**: 78 %, **m.p.** = 162 °C; **R<sub>f</sub>** = 0.30 (6.3 % methanol in DCM); **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.67 (d, *J* = 5.7 Hz, 1H), 9.48 (s, 1H), 7.56 (d, *J* = 5.7 Hz, 1H), 7.32–7.15 (m, 2H), 6.87–6.68 (m, 2H), 3.12 (t, *J* = 7.4 Hz, 2H), 1.58 (p, *J* = 7.0 Hz, 2H), 1.33–1.23 (m, 16H), 0.97–0.78 (m, 3H); **<sup>13</sup>C NMR** (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  208.1, 175.3, 161.4, 156.7, 140.7, 130.1, 123.3, 115.0, 112.2, 106.0, 41.9, 31.3, 29.0, 29.0, 28.9, 28.8, 28.7, 23.8, 22.1, 14.0; **HRMS** (ESI<sup>+</sup>) exact mass calculated for [C<sub>23</sub>H<sub>32</sub>NO<sub>4</sub>]<sup>+</sup> *m/z* 386.2326 [M+H]<sup>+</sup>, found *m/z* 386.2330 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3540, 2923, 2851, 2339, 2210, 1652, 1605, 1537, 1512, 1457, 1420, 1381, 1317, 1251, 1209, 1173, 1106, 1043, 980, 896, 832, 769, 703, 650. **4-Hydroxy-5-(4-hydroxyphenyl)-3-stearoylpyridin-2(1H)-one (13)**. Colorless solid, **yield**: 78 %, **m.p.** = 154 °C; **R<sub>f</sub>** = 0.32 (6.3 % methanol in DCM); **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.67 (d, *J* = 6.5 Hz, 1H), 9.47 (s, 1H), 7.56 (d, *J* = 6.5 Hz, 1H), 7.33–7.14 (m, 2H), 6.84–6.72 (m, 2H), 3.12 (t, *J* = 7.4 Hz, 2H), 1.58 (p, *J* = 7.0 Hz, 2H), 1.23 (m, 28H), 0.87–0.80 (t, *J* = 6.5 Hz, 3H); **<sup>13</sup>C NMR** (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  208.1, 175.3, 161.4, 156.7, 140.7, 130.1, 123.3, 115.0, 112.2, 106.0, 41.9, 31.3, 29.0, 28.9, 28.8, 28.7, 23.8, 22.1, 13.9; **HRMS** (ESI<sup>+</sup>) exact mass calculated for [C<sub>29</sub>H<sub>44</sub>NO<sub>4</sub>]<sup>+</sup> *m/z* 470.3265 [M+H]<sup>+</sup>, found *m/z* 470.3267 [M+H]<sup>+</sup>; **FT-IR**  $\tilde{\nu}$  (neat, cm<sup>-1</sup>): 3534, 2919, 2850, 2224, 1654, 1608, 1512, 1460, 1420, 1235, 1174, 1106, 988, 895, 832, 722, 638.

## 5.2 Biological Testing

AEA, *N*-(2-hydroxyethyl-1,1,2,2-*d*<sub>4</sub>)-(5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenamide, 2-AG, 2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol (CP55,940, **14**), (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(3-furanylmethyl)-5,8,11,14-eicosatetraenamide (UCM707, **15**),<sup>[18]</sup> 4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate (JZL184, **16**),<sup>[19]</sup> *N*-methyl-*N*-{[3-(4-pyridinyl)phenyl]methyl}-carbamic acid 4'-(aminocarbonyl)(1,1'-biphenyl)-4-yl ester (WWL70, **17**),<sup>[20]</sup> (S)-1-[(2*S*,3*S*)-3-hexyl-4-oxo-oxetan-2-yl]tridecan-2-yl formyl-L-leucinate (tetrahydrolipstatin, THL, **18**),<sup>[20]</sup> (5*Z*,8*Z*,11*Z*,14*Z*)-eicosatetraenoic acid, [3-(aminocarbonyl)(1,1'-biphenyl)-3-yl]-cyclohexylcarbamate (URB597, **19**),<sup>[21]</sup> 5-bromo-2-(4-fluorophenyl)-3-[4-(methylsulfonyl)phenyl]-thiophene (DuP697, **20**),<sup>[22]</sup> (9*Z*)-*N*-[1-((*R*)-4-hydroxybenzyl)-2-hydroxyethyl]-9-

octadecenamide (OMDM-2, **21**),<sup>[23]</sup> (11*R*)-2-methyl-11-[(morpholin-4-yl)methyl]-3-(naphthalene-1-carbonyl)-9-oxa-1-azatricyclo[6.3.1.0<sup>4,12</sup>]dodeca-2,4(12),5,7-tetraene (WIN55,212-2)<sup>[24]</sup> and methylarachidonylfluorophosphonate (MAFP)<sup>[39]</sup> were purchased from Cayman Chemicals Europe.<sup>[40]</sup> [Ethanolamine-1-<sup>3</sup>H]-AEA (60 Ci/mmol) and [1,2,3-<sup>3</sup>H]2-AG (20–40 Ci/mmol) were obtained from American Radiolabeled Chemicals, Inc..

### 5.2.1 Cell Culture

Human monocytic leukemia U937 cells were purchased from American Type Culture Collection (Manassas, VA) and were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 g/ml fungizone (amphotericin B), 100 units/ml penicillin, 100 g/ml streptomycin, and 2 mM L-glutamine (all from Invitrogen).<sup>[41]</sup> Cells were grown in a humidified incubator at 37 °C and in humidified atmosphere with 5% CO<sub>2</sub>.

### 5.2.2 CB1 and CB2 binding

CB1 and CB2 receptor affinities were determined using [<sup>3</sup>H]CP55,940 (0.5 nM) as displacing ligand and membrane preparations of stably transfected hCB1 and hCB2 CHO-K1 cells as previously described.<sup>[42]</sup> (*R*)-WIN55,212-2 was used as positive control (10 μM)<sup>[24]</sup> to determine total specific binding. Compounds were screened in triplicates (10 μM). K<sub>i</sub> values were calculated using the Cheng-Prusoff equation (CP55,940 hCB1 K<sub>d</sub>=0.5 nM, hCB2 K<sub>d</sub>=0.59 nM).

### 5.2.3 FAAH, MAGL and ABHD activity assays

FAAH and MAGL activity assays were performed using U937 cell homogenate and pig brain homogenate, respectively, as previously described.<sup>[36]</sup> ABHDs assay was performed using BV-2 cell homogenate as previously described.<sup>[43]</sup> Briefly, FAAH activity was assessed using 100 μg of U937 cell homogenate diluted in 200 μL of Tris-HCl 10 mM, EDTA 1 mM, pH 8 containing 0.1% fatty acid-free BSA. Compounds were added (2 μL) at the screening concentration of 10 μM and incubated for 30 min at 37 °C. Then, 100 nM of AEA-containing 1 nM of [ethanolamine-1-<sup>3</sup>H]AEA as a tracer was added to the homogenates and incubated for 15 min at 37 °C. The reaction was stopped by the addition of 400 μL of ice-cold CHCl<sub>3</sub>:MeOH (1:1), samples were vortexed and rapidly centrifuged at 16000 *g* for 10 min at 4 °C. The aqueous phases were collected and the radioactivity was measured for tritium content by liquid scintillation spectroscopy. MAGL activity was measured in a similar assay, but replacing U937 cell homogenate with 200 μg of pig brain

homogenate and using [ $^3\text{H}$ ]2-oleoylglycerol ([ $^3\text{H}$ ]2-OG) as a substrate. The reaction was performed in presence of URB597 (1  $\mu\text{M}$ )<sup>[21]</sup> in all samples to avoid the interference of FAAH activity on [ $^3\text{H}$ ]2-OG hydrolysis (approximately 10–20%). WWL70 (**17**) at 10  $\mu\text{M}$  and THL (**18**) at 20  $\mu\text{M}$  were used as positive controls for ABHD6 and ABHD12 inhibition, respectively.<sup>[20]</sup> JZL184 at 1  $\mu\text{M}$  was used as positive control for MAGL inhibition.<sup>[19]</sup>

#### 5.2.4 COX-2 activity

The experiments were performed as previously described.<sup>[32a]</sup> Briefly, the inhibition of recombinant *h*COX-2 was assessed using a COX fluorescent inhibitor screening assay kit from Cayman Chemicals Europe. Tested compounds or vehicle were pre-incubated with COX-FIS assay buffer (Tris-HCl 100 mM, pH 8), 1  $\mu\text{M}$  of COX-FIS heme *h*COX-2 FIS assay agent, and 30  $\mu\text{M}$  of ADHP (10-acetyl-3,7-dihydroxyphenoxazine) fluorometric substrate for 15 min at RT. The reaction was started by adding arachidonic acid or 2-arachidonoyl glycerol (10  $\mu\text{M}$ ). Fluorescence intensity was measured at  $\lambda = 535\text{ nm}/580\text{ nm}$  after 5 min of incubation. The results were expressed as *h*COX-2 activity.

#### 5.2.5 AEA uptake

The uptake of [*ethanolamine*-1- $^3\text{H}$ ]-AEA (60 Ci/mmol) in intact cells was investigated by using U937 cells as previously described.<sup>[16b]</sup> Briefly, approx.  $10^6$  cells were suspended in 500  $\mu\text{L}$  of serum-free medium in silanized plastic tubes and pre-incubated with a screening concentration (10  $\mu\text{M}$ ) of the compounds for 20 min at 37  $^\circ\text{C}$ . Hit compounds were tested at different concentrations in the concentration range 0.01–30  $\mu\text{M}$ . Then, the cells were incubated for 5 min at 37  $^\circ\text{C}$  with 100 nM of AEA and a small tracer (0.5 nM) of [ $^3\text{H}$ ]AEA was added. The uptake process was stopped by transferring the tubes on ice and rapidly centrifuging them at 800 *g* for 5 min at 4  $^\circ\text{C}$ . The supernatants were discarded and the pellets were re-suspended in 250  $\mu\text{L}$  of ice-cold PBS plus 1% fatty acid-free BSA and centrifuged at 800 *g* for 5 min at 4  $^\circ\text{C}$  (washing step). The washing solution was discarded and cell pellets were re-suspended in 250  $\mu\text{L}$  of ice-cold PBS and transferred into 500  $\mu\text{L}$  of a  $\text{CHCl}_3$ :MeOH mixture (1:1, v/v), vortexed vigorously, sonicated in an ice-cold water bath for 5 min, and finally centrifuged at 16000 *g* for 10 min at 4  $^\circ\text{C}$ . The organic phase was collected and transferred into scintillation tubes. The radioactivity was measured by adding 3 mL of Ultima Gold scintillation liquid (PerkinElmer Life Sciences) using a Packard

Tri-Carb 2100 TR scintillation counter (PerkinElmer Life Sciences). Data were collected from at least three independent experiments performed in triplicate, and results were expressed as intracellular [<sup>3</sup>H]AEA reduction in percentage of the vehicle-treated samples.

## Acknowledgments

Financial support by the Swiss National Science Foundation (200020\_163151) is gratefully acknowledged.

## Supplementary Information

## References

- [1] H. J. Jessen, K. Gademann, *Nat. Prod. Rep.* **2010**, *27*, 1168–1185.
- [2] a) S. Bergmann, J. Schümann, K. Scherlach, C. Lange, A. A. Brakhage, C. Hertweck, *Nat. Chem. Biol.* **2007**, *3*, 213–217; b) K. Schmidt, U. Riese, Z. Li, M. Hamburger, *J. Nat. Prod.* **2003**, *66*, 378–383; c) C. Ma, Y. Li, S. Niu, H. Zhang, X. Liu, Y. Che, *J. Nat. Prod.* **2010**, *74*, 32–37; d) S. Hayakawa, H. Minato, K. Katagiri, *J. Antibiot.* **1971**, *24*, 653–654; e) M. Shibazaki, M. Taniguchi, T. Yokoi, K. Nagai, M. Watanabe, K. Suzuki, T. Yamamoto, *J. Antibiot.* **2004**, *57*, 379–382; f) N. Hirano, J. Kohno, S. Tsunoda, M. Nishio, N. Kishi, T. Okuda, K. Kawano, S. Komatsubara, N. Nakanishi, *J. Antibiot.* **2001**, 421–427.
- [3] a) J. Tuson, *J. Chem. Soc.* **1894**, *17*, 195–197; b) L. Maquenne, L. Philippe, *C. R. Hebd. Seances Acad. Sci.* **1904**, *139*, 840–843.
- [4] a) H. L. Haller, N. E. McIndoo, *J. Econ. Entomol.* **1943**, *36*, 638; b) M. F. M. A. Bigi, V. L. V. Torkomian, S. T. C. S. de Groote, M. J. A. Hebling, O. C. Bueno, F. C. Pagnocca, J. B. Fernandes, P. C. Vieira, M. F. G. F. da Silva, *Pest. Manag. Sci.* **2004**, *60*, 933–938.
- [5] a) A. C. Ferraz, M. E. M. Angelucci, M. L. Da Costa, I. R. Batista, B. H. De Oliveira, C. Da Cunha, *Pharmacol. Biochem. Behav.* **1999**, *63*, 367–375; b) A. C. Ferraz, L. F. Pereira, R. L. Ribeiro, C. Wolfman, J. H. Medina, F. A. Scorza, N. F. Santos, E. A. Cavalheiro, C. Da Cunha, *Pharmacol. Biochem. Behav.* **2000**, *65*, 577–583.
- [6] M. Matsumoto, H. Minato, *Tetrahedron Lett.* **1976**, *17*, 3827–3830.
- [7] A. A. Alfatafta, J. B. Gloer, J. A. Scott, D. Malloch, *J. Nat. Prod.* **1994**, *57*, 1696–1702.
- [8] a) Q. Li, L. A. Mitscher, L. L. Shen, *Med. Res. Rev.* **2000**, *20*, 231–293; b) Z. Lv, C. Sheng, T. Wang, Y. Zhang, J. Liu, J. Feng, H. Sun, H. Zhong, C. Niu, K. Li, *J. Med. Chem.* **2010**, *53*, 660–668; c) P. S. Dragovich, T. J. Prins, R. Zhou, T. O. Johnson, Y. Hua, H. T. Luu, S. K. Sakata, E. L. Brown, F. C. Maldonado, T. Tuntland, C. A. Lee, S. A. Fuhrman, L. S. Zalman, A. K. Patick, D. A. Matthews, E. Y. Wu, M. Guo, B. C. Borer, N. K. Nayyar, T. Moran, L. Chen, P. A. Rejto, P. W. Rose, M. C.



- Guzman, E. Z. Dovalsantos, S. Lee, K. McGee, M. Mohajeri, A. Liese, J. Tao, M. B. Kosa, B. Liu, M. R. Batugo, J.-P. R. Gleeson, Z. P. Wu, J. Liu, J. W. Meador, R. A. Ferre, *J. Med. Chem.* **2003**, *46*, 4572–4585; d) V. Åberg, M. Sellstedt, M. Hedenström, J. S. Pinkner, S. J. Hultgren, F. Almqvist, *Biorg. Med. Chem.* **2006**, *14*, 7563–7581.
- [9] P. Schröder, T. Förster, S. Kleine, C. Becker, A. Richters, S. Ziegler, D. Rauh, K. Kumar, H. Waldmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 12398–12403.
- [10] a) E. Späth, G. Koller, *Chem. Ber.* **1923**, *56*, 2454–2460; b) D. R. Williams, S. Y. Sit, *J. Org. Chem.* **1982**, *47*, 2846–2851; c) J. H. Rigby, M. Qabar, *J. Org. Chem.* **1989**, *54*, 5852–5853; d) J. Buck, J. P. Madeley, G. Pattenden, *J. Chem. Soc., Perkin Trans. 1* **1992**, 67–73; e) D. R. Williams, P. D. Lowder, Y.-G. Gu, *Tetrahedron Lett.* **1997**, *38*, 327–330; f) Q. Zhang, A. Rivkin, D. P. Curran, *J. Am. Chem. Soc.* **2002**, *124*, 5774–5781; g) F. Ding, R. William, M. L. Leow, H. Chai, J. Z. M. Fong, X.-W. Liu, *Org. Lett.* **2013**, *16*, 26–29; h) N. R. Irlapati, R. M. Adlington, A. Conte, G. J. Pritchard, R. Marquez, J. E. Baldwin, *Tetrahedron* **2004**, *60*, 9307–9317; i) F. Schmid, M. Bernasconi, H. J. Jessen, A. Pfaltz, K. Gademann, *Synthesis* **2014**, *46*, 864–870; j) F. Ding, M. L. Leow, J. Ma, R. William, H. Liao, X.-W. Liu, *Chem. Asian J.* **2014**, *9*, 2548–2554.
- [11] a) S. H. E. Basyouni, D. Brewer, L. C. Vining, *Can. J. Bot.* **1968**, *46*, 441–448; b) A. G. McInnes, D. G. Smith, C.-K. Wat, L. C. Vining, J. L. C. Wright, *J. Chem. Soc., Chem. Commun.* **1974**, 281–282; c) K. Schmidt, W. Günther, S. Stoyanova, B. Schubert, Z. Li, M. Hamburger, *Org. Lett.* **2001**, *4*, 197–199; d) Y. Cheng, B. Schneider, U. Riese, B. Schubert, Z. Li, M. Hamburger, *J. Nat. Prod.* **2004**, *67*, 1854–1858; e) Y. Cheng, B. Schneider, U. Riese, B. Schubert, Z. Li, M. Hamburger, *J. Nat. Prod.* **2006**, *69*, 436–438.
- [12] H. J. Jessen, A. Schumacher, T. Shaw, A. Pfaltz, K. Gademann, *Angew. Chem. Int. Ed.* **2011**, *123*, 4222–4226; **2011**, *50*, 4222–4226.
- [13] P. Burch, A. Chicca, J. Gertsch, K. Gademann, *ACS Med. Chem. Lett.* **2013**, *5*, 172–177.
- [14] a) J. D. Jordan, J. C. He, N. J. Eungdamrong, I. Gomes, W. Ali, T. Nguyen, T. G. Bivona, M. R. Philips, L. A. Devi, R. Iyengar, *J. Biol. Chem.* **2005**, *280*, 11413–11421; b) J. C. He, I. Gomes, T. Nguyen, G. Jayaram, P. T. Ram, L. A. Devi, R. Iyengar, *J. Biol. Chem.* **2005**, *280*, 33426–33434.
- [15] a) Y. Dagon, Y. Avraham, G. Link, O. Zolotarev, R. Mechoulam, E. M. Berry, *Neurobiol. Dis.* **2007**, *27*, 174–181; b) F. Zhang, S. C. Challapalli, P. J. W. Smith, *Neuropharmacology* **2009**, *57*, 88–96.
- [16] a) L. De Petrocellis, V. Di Marzo, *Best. Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 1–15; b) A. Chicca, J. Marazzi, S. Nicolussi, J. Gertsch, *J. Biol. Chem.* **2012**, *287*, 34660–34682; c) C. J. Fowler, *FEBS Journal* **2013**, *280*, 1895–1904.
- [17] J. El Andaloussi-Lilja, J. Lundqvist, A. Forsby, *Neurochem. Int.* **2009**, *55*, 768–774.
- [18] M. L. López-Rodríguez, A. Viso, S. Ortega-Gutiérrez, I. Lastres-Becker, S. González, J. Fernández-Ruiz, J. A. Ramos, *J. Med. Chem.* **2001**, *44*, 4505–4508.
- [19] J. Z. Long, W. Li, L. Booker, J. J. Burston, S. G. Kinsey, J. E. Schlosburg, F. J. Pavon, A. M. Serrano, D. E. Selley, L. H. Parsons, A. H. Lichtman, B. F. Cravatt, *Nat. Chem. Biol.* **2009**, *5*, 37–44.
- [20] J. L. Blankman, G. M. Simon, B. F. Cravatt, *Chem. Biol.* **2007**, *14*, 1347–1356.
- [21] M. Mor, S. Rivara, A. Lodola, P. V. Plazzi, G. Tarzia, A. Duranti, A. Tontini, G. Piersanti, S. Kathuria, D. Piomelli, *J. Med. Chem.* **2004**, *47*, 4998–5008.

- [22] K. R. Gans, W. Galbraith, R. J. Roman, S. B. Haber, J. S. Kerr, W. K. Schmidt, C. Smith, W. E. Hewes, N. R. Ackerman, *J. Pharmacol. Exp. Ther.* **1990**, 254, 180–187.
- [23] G. Ortar, A. Ligresti, L. De Petrocellis, E. Morera, V. Di Marzo, *Biochem. Pharmacol.* **2003**, 65, 1473–1481.
- [24] D. R. Compton, L. H. Gold, S. J. Ward, R. L. Balster, B. R. Martin, *J. Pharmacol. Exp. Ther.* **1992**, 263, 1118–1126.
- [25] H. J. Jessen, A. Schumacher, F. Schmid, A. Pfaltz, K. Gademann, *Org. Lett.* **2011**, 13, 4368–4370.
- [26] F. Arndt, H. Scholz, E. Frobel, *Justus Liebigs Ann. Chem.* **1936**, 521, 95–121.
- [27] B. Kasum, R. H. Prager, *Aust. J. Chem.* **1983**, 36, 1455–1467
- [28] K. Drauz, T. Muller, M. Kottenhahn, D. Seebach, A. Thaler, *US Patent 6,090,913*, **2000**.
- [29] F. Schmid, H. J. Jessen, P. Burch, K. Gademann, *MedChemComm* **2013**, 4, 135–139.
- [30] A. Kapur, P. Zhao, H. Sharir, Y. Bai, M. G. Caron, L. S. Barak, M. E. Abood, *J. Biol. Chem.* **2009**, 284, 29817–29827.
- [31] a) M. Yu, D. Ives, C. S. Ramesha, *J. Biol. Chem.* **1997**, 272, 21181–21186; b) M. Alhouayek, G. G. Muccioli, *Trends Pharmacol. Sci.* **2014**, 35, 284–292.
- [32] a) A. Chicca, M. S. Gachet, V. Petrucci, W. Schuehly, R.-P. Charles, J. Gertsch, *J. Neuroinflammation* **2015**, 12, 1–16; b) A. Chicca, D. Caprioglio, A. Minassi, V. Petrucci, G. Appendino, O. Taglialatela-Scafati, *ACS Chem Biol.* **2014**, 9.
- [33] M. Bauer, A. Chicca, M. Tamborrini, D. Eisen, R. Lerner, B. Lutz, *J. Biol. Chem.* **2012**, 287, 36944–36967.
- [34] S. Nicolussi, J. Gertsch, in *Vitamins & Hormones*, Vol. 98 (Ed.: L. Gerald), Academic Press, **2015**, pp. 441–485.
- [35] a) P. Burch, A. Chicca, J. Gertsch, K. Gademann, *ACS Med. Chem. Lett.* **2014**, 5, 172–177; b) S. Nicolussi, J. M. Viveros-Paredes, M. S. Gachet, M. Rau, M. E. Flores-Soto, M. Blunder, J. Gertsch, *Pharmacol. Res.* **2014**, 80, 52–65.
- [36] S. Nicolussi, A. Chicca, M. Rau, S. Rihs, M. Soeberdt, C. Abels, J. Gertsch, *Biochem. Pharmacol.* **2014**, 92, 669–689.
- [37] W. C. Still, M. Kahn, A. Mitra, *J. Org. Chem.* **1978**, 43, 2923–2925.
- [38] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, 29, 2176–2179.
- [39] D. G. Deutsch, R. Omeir, G. Arreaza, D. Salehani, G. D. Prestwich, Z. Huang, A. Howlett, *Biochem. Pharmacol.* **1997**, 53, 255–260.
- [40] For a review on tool compounds for the investigation of lipid metabolism, please refer to C. Doler, M. Schweiger, R. Zimmermann, R. Breinbauer, *ChemBioChem* **2016**, 17, 358–377.
- [41] a) K. C. M. Verhoeckx, S. Bijlsma, E. M. de Groene, R. F. Witkamp, J. van der Greef, R. J. T. Rodenburg, *Proteomics* **2004**, 4, 1014–1028; b) C. A. Izeboud, R. M. Vermeulen, A. Zwart, H. P. Voss, A. S. v. Miert, R. F. Witkamp, *Naunyn Schmiedeberg's Arch. Pharmacol.* **2000**, 362, 184–189; c) V. K. Nagaleekar, G. Sabio, I. Aktan, A. Chant, I. W. Howe, T. M. Thornton, P. J. Benoit, R. J. Davis, M. Rincon, J. E. Boyson, *J. Immunol.* **2011**, 186, 4140–4146.
- [42] A. Chicca, D. Caprioglio, A. Minassi, V. Petrucci, G. Appendino, O. Taglialatela-Scafati, J. Gertsch, *ACS Chemical Biology* **2014**, 9, 1499–1507.
- [43] A. Chicca, J. Marazzi, J. Gertsch, *Br. J. Pharmacol.* **2012**, 167, 1596–1608.

